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ERRATA AND AUTHORS' EMENDATIONS

Page 4, explanatory legend for Plate 1, line 6, delete "1900" after "April."
Page 4, explanatory legend for Plate 1, line 7, "Buitenzord, 1898," should be "Buitenzorg 1899."
Page 5, footnote 13, line 2, "National" should be "Mycological."
Page 5, explanatory legend for Plate 2, lines 1 and 2, "ascomata" should be "ascoma."
Page 7, line 28, "this" should be "the present." and "or" should be "and."
Page 7, explanatory legend for Plate 3, G, lines 5 and 6, "2" should be "3" and "30" should be "32."
Page 7, explanatory legend for Plate 4, A–C, line 4, "2" should be "11."
Page 9, footnote 21, "Yoshinga" should be "Yoshinaga" and "2" should be "11."
Page 15, line 34, "intercepted" should be "samples from."
Page 15, Table 1, last column, "Containers in shipments from scabbed samples" should be "Containers in shipments from which scabbed samples were taken."
Page 20, explanatory legend for Plate 4, C, *a*, "perithecia" should be "ascoma."
Page 21, explanatory legend for Plate 5, A, *a*, "Perithecium" should be "Ascus."
Page 22, under Literature Cited, No. 9, "1930" should be "1931."
Page 32, Table 4, footnote a, line 1, "23 per cent" should be "36 per cent."
Page 35, footnote 1, line 1, "Aug. 25, 1931" should be "Aug. 25, 1930" and "January 1930" should be "January 1931."
Page 36, Table 1, under "Method of preparation," column 3, line 22, "17° C." should be "−17° C."
Page 39, Table 4, last column, under "Final condition," line 6, "3, scurvy" should be "2, scurvy."
Page 96, Table 4, experiment 2, column 8, reference letter "c" should be before + mark on line below.
Page 97, Table 5, column 1, lines 11, 12, and 19, "Na_2CO_3" should be "NaCl."
Page 126, legend for Figure 2, line 4, "$0.002088X^2$" should be "0.002088X."
Page 126, legend for Figure 2, line 5, "0.00214^2X" should be "0.002142X."
Page 127, Table 2, column 7, under "Per cent of birth weight," line 8, "260.57" should be "160.57."
Page 132, Table 4, column 5, under "Born alive," line 1, "82.84" should be "82.34."
Page 133, nineteenth line from bottom, "50.145" should be "50.514."
Page 133, eighteenth line from bottom, "0.2641" should be "0.2341."
Page 135, Table 6, column 1, line 5, "females" should be "males."
Page 147, legend for Figure 11, line 3, delete "7" before "months."
Page 156, line 24, "0.60" should be "0.66."
Page 157, line 3, "0.09" should be "0.08."
Page 169, Table 2, last column, line 15, "25.23" should be "0.25."
Page 247, seventh line from bottom, "Kettendofer's" should be "Pettenkoffer's."
Page 298, line 11, "first" should be "second."
Page 317, line 11, "slightly" should be "moderately."
Page 481, eleventh line from bottom, "distinct" should be "indistinct."
Page 530, Table 4, last column, line 6, ".067" should be ".064."
Page 530, footnote b, line 2, "or the remaining 95 bolls" should be "*r* for the remaining 95 bolls."
Page 654, footnote b, after "½ c. c." insert "of spleen suspension."
Page 654, footnote d, after "½ c. c." insert "of culture suspension."
Page 716, Table 1, column 1, "Summer varieties[a]" should be "Varieties from Pacific Northwest."
Page 716, footnote [a] should be deleted.
Page 765, footnote 7, "4 : 10" should be "4 : 1."
Page 793, twenty-first line from bottom, "negative" should be "native."
Page 810, lines 27 and 28, delete "of the" between "crushing" and "strength."
Page 813, Table 2, column 8, line 5, under "Hot water extractives" "13.52" should be "13.62."
Page 816, Table 2, last column, under "Base multiplied by value," "sq. in." should be "cu. in."
Page 820, Table 2, last column, under "Base multiplied by value," "sq. in." should be "cu. in."
Page 862, Table 5, column 1, under "Symbol," lines 1 and 2, "*rjG*" should be "*rJG*" and "*dG*" should "*rIG*."
Page 885, second line of text above Table 4, delete "the" before "net energy."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 42 WASHINGTON, D. C., JANUARY 1, 1931 No. 1

SCAB OF CANAVALIA CAUSED BY ELSINOE CANAVALIAE[1]

By ANNA E. JENKINS

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INTRODUCTION

In connection with investigations of diseases caused by members of the form genus Sphaceloma De Bary (*4*)[2] and the ascomycetous genus Plectodiscella Woronichin (*44, p. 232*), the writer has had occasion to make a study of a little-known disease of Canavalia (Canavalia DC.), including the morphology and the status of the classification of its causal fungus. This study has involved an examination of certain other myriangioid fungi than those referred to above. As supplementing those already available, most of which are mentioned or cited in this account, the data thus obtained are here given for reference in connection with these investigations but primarily because of their direct bearing on the Canavalia disease and on a destructive disease of Lima bean (*Phaseolus lunatus macrocarpus* Benth.) now being investigated (*19*). The work is based upon material contained in the Mycological Collections of the Bureau of Plant Industry and in the United States National Herbarium.

SPECIES OF CANAVALIA AFFECTED

The disease of Canavalia here discussed has been reported as affecting two species of this genus, *C. gladiata* (Jacq.) DC. of Asiatic origin (*28*) and *C. ensiformis* (L.) DC. which "it is practically certain * * * is native to America." According to Piper (*28*) the former species is commonly known as sword or saber bean and the latter as jack or horse bean, and the two have been greatly confused. Raciborski (*29, t. 1, p. 14*),[3] who, so far as known, discovered as well as first reported the disease about 30 years ago, noted it as affecting the sword bean, that is, *C. gladiata*.

In the four reports of the disease from Ceylon that have been found it is listed once as affecting "sword bean," no scientific name being given (*25*), and once as affecting "*Canavalia ensiformis*" with no mention of a common name (*26*); in the two other reports one or both common names, sword and saber bean, are given and also the binomial *Canavalia ensiformis* (*7, 8*). Practically the same situation is found in the four Philippine reports of the disease which are available (*2, 3, 31, 43*). Thus there exists here the confusion referred to by Piper. In the case of the reports of the disease from

[1] Received for publication July 21, 1930; issued January, 1931. This is a somewhat amplified section of a paper read before the Botanical Society of America, Mycological Section, Dec. 30, 1929. The illustrations are by J. F. Brewer.

[2] Reference is made by number (italic) to Literature Cited, p. 10.

[3] Buitenzorg, Java. M. Raciborski. (RACIBORSKI, M. CRYPTOGAMAE PARASITICAE IN INSULA JAVA LECTAE EXSICCATAE. Fasc. 1, No. 9. Buitenzorg. 1899.)

Ceylon it would seem that the host concerned is *C. gladiata*. Increasing the uncertainty of the exact species of Canavalia affected in the Philippines is the information recently received from E. D. Merrill [4] that the "*C. ensiformis* of most Philippine authors appertains to the endemic *C. luzonica* Piper * * *." It was learned from the same source that "*C. gladiata*" and "*C. ensiformis*" are "not common and * * * never spontaneous in the Philippines," where the latter or West Indian species was apparently "introduced * * * after the American occupation." Thus, although *C. ensiformis* has been listed as susceptible to the Canavalia disease here discussed, it is not definitely known at present whether this species is actually affected. The material in the United States National Herbarium referred to consists of the terminal part of a main stem or branch and one empty pod of Canavalia collected in the Singapore Botanic Gardens in April, 1921. This specimen, recently found by the writer to be severely affected by the disease, was labeled *C. ensiformis* when acquired, but it had later been identified as *C. gladiata*(?) by Piper. Had seeds been available it is probable that he would not have questioned his specific identification of this specimen.

THE DISEASE

NAME

In the small amount of literature concerning the disease under discussion there is no mention of a name by which it may be designated. On this account, and because of its similarity to the rutaceous disease commonly known as citrus scab, this legume disease is here referred to as scab of Canavalia.

HISTORY, RANGE, AND IMPORTANCE

As already indicated, scab of Canavalia, so far as known, was discovered in Java by Raciborski about 30 years ago and has since been observed in the Philippines and Ceylon. Based upon the scabbed condition of the specimen from Singapore found by the writer in the United States National Herbarium, its range is now known to include the Malay Peninsula. Other than notations of its occurrence in Ceylon and in the Philippines and the two brief accounts by Raciborski (*29, t. 1, p. 14*) and Arnaud (*1, p. 685–686*) dealing mainly with its leaf symptoms, the disease has scarcely been investigated and little definite information is available concerning its importance. Raciborski reported that in Java it affects leaves, stems, and fruits, producing swellings that resembled those due to Exoascaceae. Baker (*3, p. 159*) stated that in the Philippines it caused "widespread distortion and death of both leaves and stems." This disease is probably one of the most important of those affecting Canavalia species susceptible to its attack.

SYMPTOMS

Raciborski stated that on fresh leaves (*29, t. 2, p. 3*) the lesions measured 2 to 12 mm. in diameter by about 1 to 3 mm. in thickness. Their general appearance as further described by him is represented by the Raciborski specimen already cited and by a specimen from

[4] Letter dated March 10, 1930.

the Philippines collected by Baker [5] in 1913, illustrations of which are shown in Plate 1, A–C. These illustrations show, in addition, the shot-hole effect apparently resulting from the falling out of the lesions. Lesions not noticeably swollen are visible on the midrib and veins of one of the leaves. (Pl. 1, C.)

The leaf lesions on the herbarium specimens from Singapore are generally brighter in color and larger than those described by Raciborski. These lesions are here bone brown [6] at the center, varying to Mars brown toward the outside. The scattering, more or less circular pod lesions, which are generally flat but occasionally slightly raised or depressed, show practically the same coloration as the leaf lesions. The numerous stem lesions, which are circular, elliptical, or occasionally elongated, are paler, that is, cinnamon brown, somewhat grayed. The circular leaf lesions are about 6 mm. across; others following the veins attain a length of 2 cm. and a width of 4 mm. The larger pod lesions are about 3 mm. in diameter, while the stem lesions may extend for a distance of 2 cm. along the shoot, enveloping more than half its circumference. The histologic structure of the galls (pl. 1, D) as studied by Raciborski, Arnaud, and the writer appears to be homologous with that of the leaf lesions of citrus scab as recently studied by Cunningham (*9*). In other words, the 2-layered development reported by Arnaud undoubtedly corresponds to what in leaf lesions of citrus scab Cunningham designates as the formation of a "phellogen in the spongy parenchyma * * * as a result of" whose activity "* * * a definite phellem is laid down, thus completely isolating the portion invaded" by the causal fungus, *Sphaceloma fawcettii* Jenkins. The gall formation was here due to an increase in the number as well as in the size of the cells in the spongy parenchyma.

THE PATHOGENE

MORPHOLOGY

Hyphae

The hyphae of the genus Elsinoe, in which is classified the causal fungus of scab of Canavalia, are described (*29, t. 1, p. 14*) as intercellular and as forming a thick, colorless or light-gray pseudoparenchyma between the epidermis and the mesophyll, which remains covered by the epidermis for a considerable period. Those of the pathogene are said (*29, t. 1, p. 14*) to be barrel-shaped and the stromatic layer to range from 10μ to 38μ in thickness. In its orientation Arnaud (*1, p. 685 and pl. 3, C*) observed that the stromatic layer is found just outside the diseased tissue here termed the "phellem" and that it also grows into the outer cells of this tissue as well as beneath the disrupted epidermis.

Imperfect Stage

Neither Raciborski nor Arnaud reported a conidial stage for the pathogene of scab of Canavalia, but such a stage doubtless exists. In fact, small, spherical, hyaline bodies, interpreted as microconidia

[5] Mount Maquiling, near Los Baños, Province Laguna Luzon, Philippines, 1913, C. F. Baker. (Baker, C. F. Fungi Malayana. Century 1, No. 26.) Specimen in Mycological Collections of the Bureau of Plant Industry.

[6] The color readings in this account were made by J. Marion Shull and are based on the following publication: Ridgway, R. Color standards and color nomenclature, 43 p. illus., Washington, D. C. 1912.

of the Elsinoe, were seen by the writer on some of the material examined. This consists of the specimens from Java, Singapore, and the Philippines already cited, as well as an additional Philippine specimen collected by Baker [7] in 1914. The microconidia were similar to those recently reported by the writer (*17*) for *Sphaceloma symphoricarpi* Barrus and Horsfall, in which form genus, created by De Bary (*4*), the conidial stage of this fungus is certainly to be classified. Acervuli are probably present on the material examined, but a critical search has not been made for them. The microconidia seen were possibly produced as sprout conidia from ascospores. In Plate 3, C, *a*, are shown similar although somewhat larger structures interpreted as microconidia of *Uleomyces sanguineus* (Speg.) Syd.[8] (*38, p. 219*), which is the type of the genus Uleomyces P. Henn. (*12, p. 107*), referred to later. These indicate a similar conidial stage for this related species not hitherto noted.

Perfect Stage

Raciborski (*29, t 1, p. 14*) and Arnaud (*1, p. 685, and pl. 3, C*) reported that the single layer or groups of asci in the causal fungus of scab of Canavalia develop in the stromatic layer already mentioned. (Pl. 1, D.) Raciborski gave the measurements of the globular asci and of the transversely septate hyaline spores, eight in an ascus, as 16μ–19μ by 20μ–22μ and 9μ–12μ by 2.8μ–3.5μ, respectively. Upon discovering that the spores in the Javanese material collected by Raciborski [9] were not colored and muriform as in Uleomyces, where, as will later be more fully explained, Arnaud classified the fungus, he attributed this condition to the immaturity of the specimen. Longitudinal septation might be delayed as in *Uleomyces sanguineus*, he explained.

On the specimen from Singapore there were seen both asci and spores judged to be more mature than those described by Raciborski or than those seen by Arnaud. The largest asci, which were here clavate and furnished with a short stipe, attained a size of 24μ–30μ, while the largest spores, some of them still within the ascus, measured 28μ by 9μ. The more mature spores ranged from hyaline to yellowish or greenish yellow. The transverse walls were well defined, but longitudinal septation, which Arnaud stated might be delayed, was not seen. In the thickened apical region the walls of a number of the asci were faintly stained, apparently from the coloring matter in the surrounding matrix. Petch (*27, p. 62–63*) has made a similar observation in the case of *Myriangium duriaei* Mont. and Berk. (*6, p. 73*), the type of the genus Myriangium Mont. and Berk. (*6, p. 72–73*), on which was founded the order Myriangiales Starbäck [10] (*35*), referred to in the following section. Here, not only the ascus wall but also the spores

[7] Los Baños, Province Laguna, Luzon, Philippines, 1914, C. F. Baker. (Sydow. Fungi exotici exsiccati. Fasc. 9, No. 420.) In Mycological Collections of the Bureau of Plant Industry.

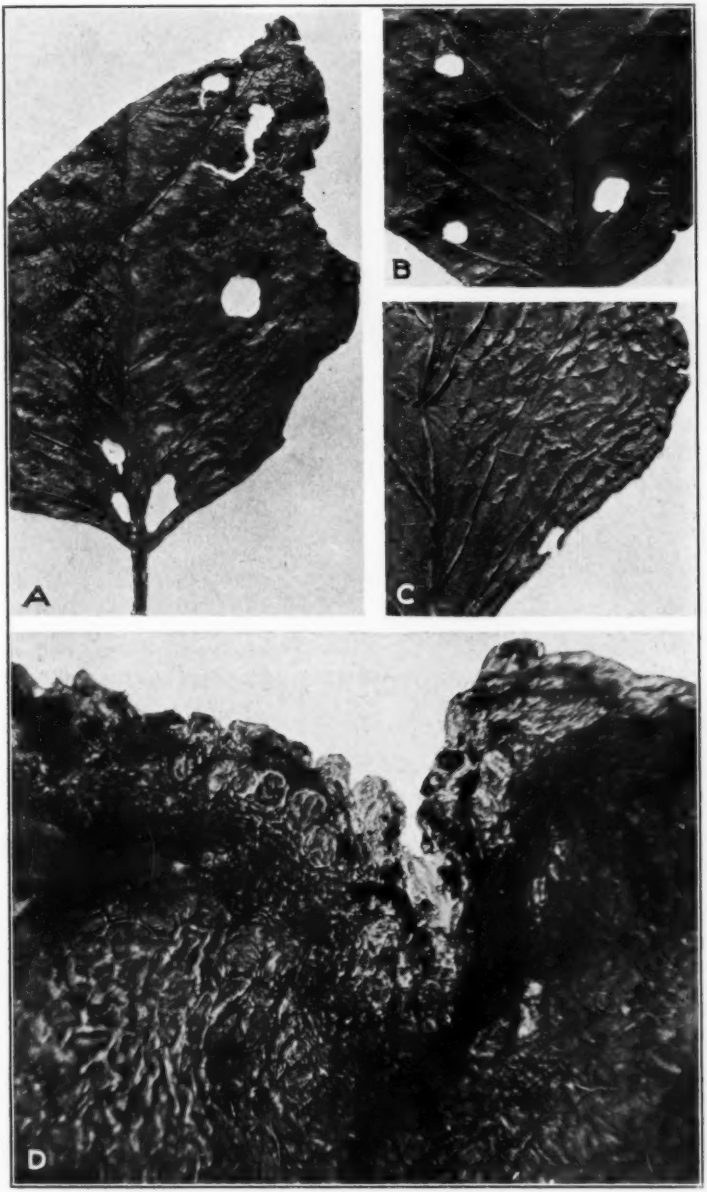
[8] Synonyms: *Uleomyces parasiticus* P. Henn. (*12, p. 107*), *Cookella parasitica* (P. Henn.) P. Henn. (*13, p. 275*), and others referred to later.

[9] Op. cit. (See footnote 3.)

[10] Synonym: Myriangiacei Nylander (*23, p. 139*).

EXPLANATORY LEGEND FOR PLATE 1

Leaves of Canavalia infected by *Elsinoe canavaliae*. Upper (B) and lower (A and C) leaf surfaces, and gall (D) in median cross section, showing stromatic fertile layer, shot-hole effect, and, particularly in C, lesions on midrib and veins. A–C, × 1; D, × 380. Material for A and B collected by C. F. Baker, Mount Maquiling, near Los Baños, Province Laguna, Philippines, 1913 (C. F. Baker, Fungi Malayana Cent. 1, No. 26); specimen in Mycological Collections of the Bureau of Plant Industry. Material for C and D collected by M. Raciborski, Buitenzord, Java, April, 1900 (M. Raciborski, Cryptogamae Parasiticae in insula Java lectae exsiccatae Fasc. 1, No. 9, Buitenzord, 1898)



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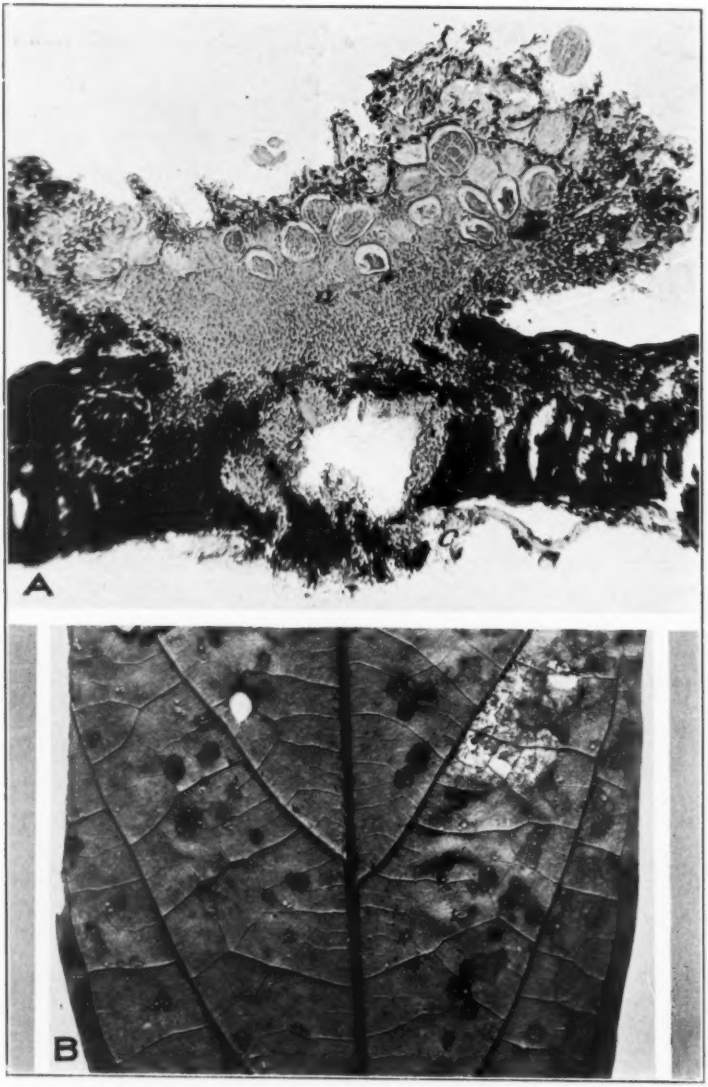


FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

within the ascus were stained brown, as it seemed, from the "adherent * * * decaying" stroma. So far as the writer knows, only hyaline spores had previously been reported for this species. It may be mentioned here that only the supposed lack of colored spores in Myriangium prevented Hennings (*14, p. 354*) in 1901 from transferring to this genus his *Uleomyces parasiticus*, i. e., *U. sanguineus*, at the time he recognized its identity with *Phymatosphaeria sanguinea* Speg.[11] (*34, p. 57*). This would have made the genus Phymatosphaeria Speg. (*34, p. 57*) a synonym under the older genus Myriangium. In the classification of Theissen (*40, p. 312*) and Theissen and Sydow (*41, p. 439*), the genera Uleomyces and Myriangium are separated on the basis of the distribution of the asci in the ascoma.

NAME, HISTORY, AND CLASSIFICATION

Scab of Canavalia is caused by *Elsinoe canavaliae* Rac. (*29, t. 1, p. 14*). This fungus is the type of the genus Elsinoe Rac. (*29, t. 1, p. 14*). Raciborski classified the genus and therefore the species in the family Exoascaceae near Magnusiella. In 1909, in connection with his revision of the order Myriangiales, or family Myriangiacei, as he treated the group, Von Höhnel (*16, p. 349–376*) removed this genus and species from the Exoascaceae, raised to generic rank Myriangina,[12] formerly classified as a subgenus of Myriangium by its author, Hennings (*15, p. 55*), and then erected for them the new family Elsinoëen. This new family he considered to be of doubtful systematic position. *Myriangina mirabile* he stated might be regarded as an ingrown form of the Plectascales, while in his opinion the genus Elsinoe afforded a transitional form not theretofore reported in that order. He concluded that the relationships of the new family might be with the Plectodiscales or the Protodiscales, but that the final solution of its classification rested upon transitional forms as yet undiscovered.

Myriangina mirabile is based upon material collected in Brazil in 1901,[13] which has been examined by the writer. For comparison with *Elsinoe canavaliae*, illustrations from material found in Brazil on leaves of a species of Lauraceae,[14] (on which host the fungus was originally described) and on leaves of an undetermined host [15] are here shown in Plate 2 and in Plate 4, D–G. Plates 2, B, and 4, D, show the general appearance of the ascomata in this species. The shot-hole effect in the leaf shown in Plate 4, D, resembles that here illustrated for scab of Canavalia. (Pl. 1, A–C.) In one of the sections similar to that shown in Plate 2, A, asci were scattered about in the stroma within the leaf tissue (pl. 2, A, *b*), as well as in that composing the superficial ascoma. It will be noted that in this species the ascoma may protrude

[11] Synonym: *Ascomycetella sanguinea* (Speg.) Sacc. (*33, p. 847*).
[12] Synonym: Dictyomollisia Rehm, typified by *D. albidogranulata* Rehm (*39, p. 403*).
[13] Sao Paulo, Brazil, April, 1901, A. Puttemans. (PUTTEMANS. A., FUNGI S. PAULENSIS No. 176.). Specimen in United States National Herbarium (cited in *15, p. 55*).
[14] Sao Paulo, Brazil, 1901, F. von Höhnel. (REHM, H. ASCOMYCETES. Fasc. 33, No. 1704) (*30, p. 298*).
[15] Sao Leopoldo, Brazil, 1908, F. Theissen. (THEISSEN, F. DECADES FUNGORUM BRASILIENSIUM. Century 1, No. 75, labeled *Myriangium* (*Myriangina*) *mirabile* P. Henn.). (*42, p. 403.*)

EXPLANATORY LEGEND FOR PLATE 2

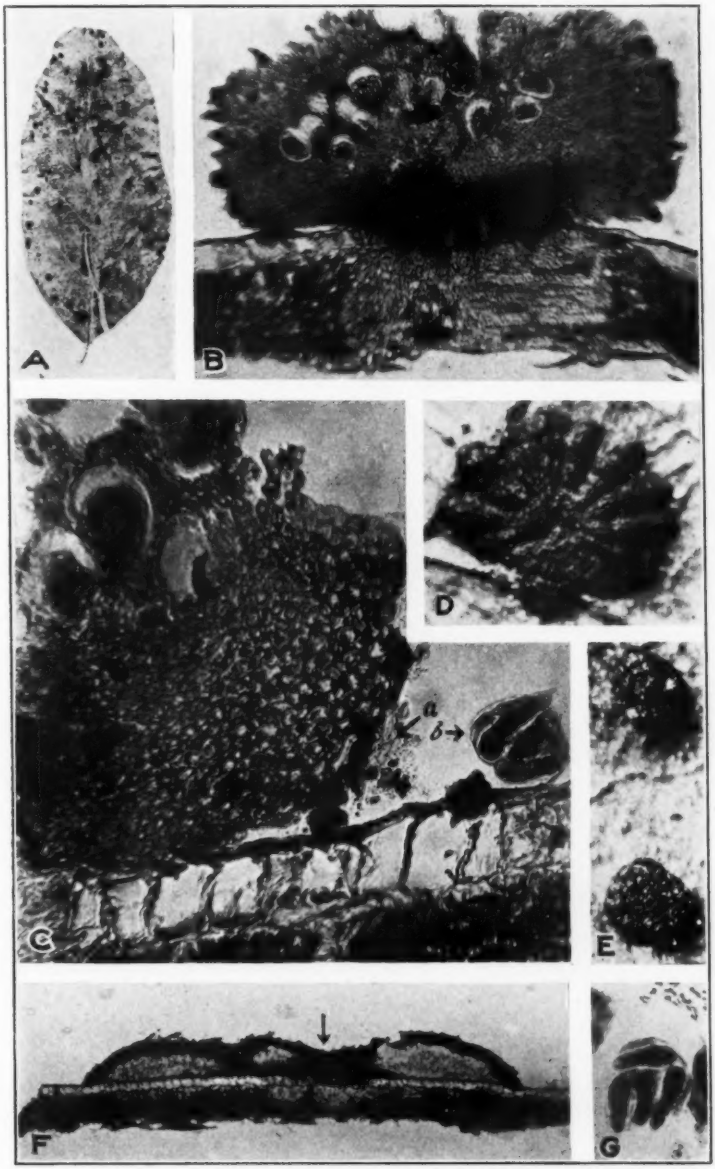
Myriangina mirabile on leaf of Lauraceae: A, Ascomata in median cross section; *a* ascomata protruding from lower leaf surface; *b*, ascomata within leaf tissue; *c*, remnants of ascomata protruding from upper leaf surface. ×380. B, Ascomata in situ on lower leaf surface. ×1. Material collected by F. Theissen, Sao Leopoldo, Brazil, 1908 (Theissen, Decades Fungorum Brasailiensium, Cent. 1, No. 75). (*39, p. 403*)

on either surface of the leaf. (Pl. 2, A, *a* and *c*.) The scantiness of the stroma in *Elsinoe canavaliae*, as compared to its abundance in *Myriangina mirabile*, was held by Von Höhnel (*16, p. 373*) to account for the asci being smaller and less numerous in the former species than in the latter. The muriform spores of *M. mirabile* may be seen in Plates 2, A, and 4, F, while in Plate 4, G, is shown the 2-layered ascus apparently not previously reported for this genus or species. Here the "primary membrane" (pl.4, G, *a*) as so interpreted by Millardet (*22, p. 15, pl. 3, fig. 29, b*) for *Myriangium duriaei*, has become separated from what by the same author is termed the "secondary layer" (pl. 4, G, *b*) of the ascus in Myriangium. With somewhat different explanations the 2-layered ascus has been reported and illustrated by Miles (*21, p. 78–79, fig. 1, a*) for *M. tuberculans* Miles, by Petch (*27, p. 61–63, pl. 2, figs. 14 and 20*) for *M. duriaei*, and by Stevens and Weedon (*36, pl. 19, fig. 9 and pl. 20, fig. 15*) for the two myriangioid species *Kusanoopsis guianensis* and *Myrianginella tapirae*, of which they are the authors. Stevens and Weedon remark upon the rarity of the double-walled ascus in the ascomycetes in this connection, citing De Bary's (*5, p. 93–96, figs. 46 and 47*) account of the homologous structure in the ascus in *Sphaeria scirpi* and *Pleospora herbarum*. The conidial stage of *Myriangina mirabile*, not hitherto reported, is apparently similar to that of *Elsinoe canavaliae*. Microconidia of this species, sprouted from ascospores still inclosed within the secondary layer of the ascus, are here shown in Plate 4, G. Other conidia were seen which may belong to this fungus. These were hyaline or reddish brown, minute and spherical or somewhat larger and elongate, resembling those illustrated by the writer [16] for *Sphaceloma fawcettii* Jenkins.

The two genera Elsinoe and Myriangina are certainly to be classified in the Myriangiales, as the group was recognized by Theissen (*40, p. 311*) in 1916, and where they were placed by Theissen and Sydow (*41, p. 433–447*) in their arrangement of the order published the year following. These authors erected for the Elsinoeae, as they termed Von Höhnel's family Elsinoeen, and the family Plectodiscelleae Woronichin (*44, p. 232*) the suborder Protomyriangieae. The other suborder in the Myriangiales as considered by them is that of Eumyriangieae, treated by its author Theissen (*40, p. 312*) as a subfamily in this order. Both Theissen (*40, p. 312*) and Theissen and Sydow (*41, p. 439*) placed the genera Myriangium and Uleomyces in this subfamily or suborder, separating them from each other as already indicated. Theissen and Sydow (*41, p. 437*) state that in the Protomyriangieae the ascoma is intramatrical and not definitely limited in extent, while in the Eumyriangieae it is definitely formed and erumpent with a differentiated surface.

In Arnaud's (*1, pp. 672–706*) more recent revision of the Myriangiales the order comprises two families, the Myriangiaceae with the two tribes Cookellées [17] and Myriangiées, and the Saccardinulacées. As here indicated,[17] the Elsinoeen are made by Arnaud (*1, p. 679*) a synonym of his tribe Cookellées. In this tribe he (*1, p. 676*) includes the genus Uleomyces and one other, Cookella, making Elsinoe, Plecto-

[16] JENKINS, A. E. DEVELOPMENT OF THE CITRUS-SCAB ORGANISM, SPHACELOMA FAWCETTII. Unpublished.
[17] Synonyms: Cookellacées Von Höhnel pro parte, Saccardiacées Von Höhnel, Elsinoées Von Höhnel, and Plectodiscellées Woronichin (*1, p. 681*).



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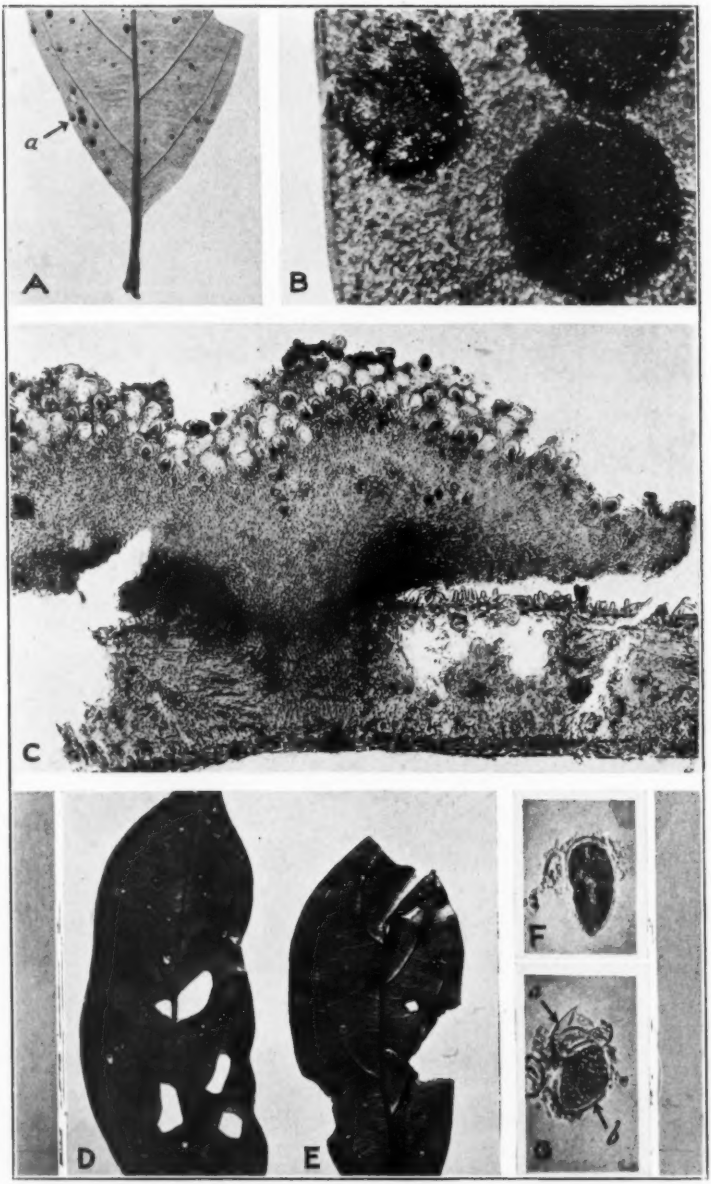

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discella, Myriangina, and Dictyomollisia synonyms of Uleomyces and definitely transferring *Elsinoe canavaliae* to that genus (*1, p. 685*). The two species representing the monogeneric family Plectodiscelleae are also transferred. Formerly known as *Plectodiscella piri* Woronichin and *P. veneta* (Sacc.) Burkholder, they thus became *Uleomyces piri* (Woronichin) Arnaud and *U. veneta* (Burkholder) Arnaud. The first causes an important disease of apple and pear in Europe, not known to occur in this country (*18, 20, 24, 44*), and the second the widespread anthracnose of brambles (Rubus).

Although Elsinoe has been relegated to synonymy under Uleomyces and the type definitely transferred to the latter genus, it has seemed desirable here to continue to refer to both genus and species by their original names. When more data are available it is planned to discuss further the synonymy of these and the other closely related genera here mentioned and their type species. The asci and spores in the type species of the genus Uleomyces (pl. 3, C, *b*, and G) are considerably larger than in that of Elsinoe, measuring 60μ–100μ by 30μ–50μ, and 22μ–32μ by 11μ, respectively (*12, p. 107*), and are described by Spegazzini (*34, p. 57*) as hyaline and by Hennings (*12, p. 107*) as brown or deep wine color. That the latter color may be due to a staining from the bright coloration of the stroma is indicated by an observation recorded in the legend of Plate 3.

The families Myriangiaceae and Saccardinulaceae, as well as the two tribes of the former family, are separated by Arnaud (*1, p. 678*) on the same basis, namely, pathogenicity and characteristics of the ascoma. As he states in his key (*1, p. 678*), the Saccardinulaceae are pathogenic on phanerogams and the Myriangiaceae on other fungi or on insects. Previously in this account, or in the following section, it is indicated that the type species of the genus Uleomyces or those of the genera relegated to synonymy under this genus are actually pathogenic on phanerogamic plants, as is also apparently *Cookella microscopica*, the only species that Arnaud includes in the genus Cookella. The type species of the genus Myriangium was originally reported by Montagne and Berkeley (*6, p. 73*) on the phanerogamic host *Morus albus*, and Millardet (*22, p. 13*) found the species to be

EXPLANATORY LEGEND FOR PLATE 3

Uleomyces sanguineus and Parmularia styracis on leaves of Styrax.

A.—General appearance of fructifications, most of which are of *Uleomyces sanguineus*. × 1

B, C.—*Uleomyces sanguineus* in median cross section, together with microconidia (C, *a*) and ascospores (C, *b*) as originally contained in an ascus. B, × 130; C, × 380

D, E.—Enlarged entire fructifications of *Parmularia styracis* and *Uleomyces sanguineus*, respectively. × 22

F.—Median cross section through the Parmularia fruit body shown in D, the arrow indicating the central part of this rosettelike structure. × 44

G.—An individual ascus of *Uleomyces sanguineus* in situ in the stroma in which it was borne. × 380. After the sections had been in mounting fluid for a time they became surrounded by a halo of purplish coloring matter diffusing from the ascomata, and the spores, which were mostly reddish brown when the mounts were made, became stained a deep purple

Material collected by J. Rick at São Leopoldo, Brazil, in 1904 (Rick, Fungi Austro-Americani, Fasc. 2, No. 49). (*30, p. 309*)

EXPLANATORY LEGEND FOR PLATE 4

A–C.—Ascomata of *Uleomyces decipiens* on lower surface of leaf of *Pasania glabra*. A, × 1; B, enlargement of A, *a*, × 22; C, median cross section through one of the ascomata illustrated in B, showing its direct attachment to the leaf, × about 100. Material collected by T. Yoshinaga, Japan, 1921 (Sydow, Fungi Exotici Exsiccati, Fasc. 2, No. 513). (*37*)

D–G.—Upper (D) and lower (E) surface of leaf of undetermined host attacked by *Myriangina mirabile*, showing shot-hole effect resulting from the falling out of the lesions, and the white ascomata of this fungus on the upper leaf surface. × 1. F and G, Two asci, in one of which (G) microconidia have sprouted from the spores, giving them a granular appearance, and in which the primary membrane (*a*) of the ascus has become separated from the secondary layer (*b*). × 380. Material collected by F. von Höhnel[illegible] fio Paulo, Brazil, 1901 (H. Rehm, Ascomycetes, Fasc. 39, No. 1704). (*30, p. 208*)

definitely pathogenic on *Nerium oleander*. According to Arnaud, it was Zimmermann (*45, p. 876*) who first indicated "assez timidement" that *Myriangium duriaei* was parasitic on a scale insect. Zimmerman stated that the Myriangium grew within and on the surface of *Ichnapsis filiformis*, occurring on leaves of *Coffea liberica* and Elias in Java. He explained, however, that the fungus was not joined to the body of the Ichnapsis and that perhaps it penetrated only the scale covering of the dead insect. His illustrations suggest that, although the insect and the fungus were closely associated, the latter may have been growing directly on the phanerogamic substrata.

Assuming the direct pathogenicity on phanerogams of the fungi mentioned above, this would leave only the criterion of mycological differences, that is, characteristics of the ascoma, to distinguish the families, tribes, and, to a certain extent, even the genera composing the Myriangiales of Arnaud. Whether the differences in the fructifications are sufficient to warrant the formation of more than one family or tribe in the order is debatable. Further knowledge of the group will assist in elucidating these points.

Nylander (*23*) placed the family Myriangiaceae among the lichens, but it was definitely recognized as belonging to the fungi by Millardet (*22*) in 1870. That author stated that the affinities of the group appeared to be with the Tuberaceae and that it should receive equal ranking with this group. Spegazzini (*34, p. 57*), creating the family Phymatosphaeriaceae, typified by *Phymatosphaeria sanguinea*, i. e., *Uleomyces sanguineus*, also indicated that the relationships of that family were with the Tuberaceae.[18] In a schematic arrangement, however, Millardet actually placed the Myriangiaceae between the family Onygenaceae and the Pyrenomycetes. The Onygenaceae are a family of the Plectascineae of Fischer (*11*), who appended to this order the family Myriangiaceae, then including only the genus Myriangium. In Montagne and Berkeley's (*6*) original report of *M. duriaei* it was suggested that the organism resembled Dothidea as well as certain lichens. In 1909 Von Höhnel (*16, p. 351*) placed the family Myriangiaceae in the Dothideales, near which order the family had been placed by Starbäck (*35*) in 1899, when he raised it to ordinal rank. Theissen and Sydow (*42, p. 6*) placed the order Myriangiales in the Dothidineae, containing the two other orders Dothideales and Pseudosphaeriales. Arnaud (*1, p. 653, 677*) stated that the order appeared to consist of primitive pyrenomycetes possibly derived from the Plectascales. He placed the order before the Microthyriales, with which he considers the Dothideales have developed in a parallel line.

PATHOGENICITY

In his brief description of the genus Elsinoe, Raciborski stated that its members consisted of parasitic ascomycetes producing galls on the host plants. Such parasitism for *Elsinoe canavaliae* is not doubted by the present writer. Arnaud, after examining the material of *E. canavaliae* collected in Java by Raciborski,[19] indicated that the organism was here growing on another fungus within the galls rather than being the direct cause of these malformations. In one statement, however, he suggested that the galls were actually caused by *E. canavaliae*, or, as he termed the fungus, "*Uleomyces*

[18] Tuberineae Fischer (*10*).

[19] Op. cit. (See footnote 3.)

canavaliae." His assumption of the parasitism of the organism on another fungus and not on Canavalia may have been influenced in part at least by the fact that Hennings (*12, p. 107*), in describing the type species of the genus Uleomyces, i. e., *U. sanguineus*, or, as termed by him, "*U. parasiticus*," had stated that it was parasitic on the ascomycete *Parmularia styracis*, which occurs on Styrax. Hennings based his description on a specimen gathered in Brazil in 1892 by Ule, in whose honor the genus is named. As already indicated, he later recognized that the same organism, discovered in Paraguay by Balansa in 1883, had been described by Spegazzini under the name *Phymatosphaeria sanguinea*. In describing the fungus under this name Spegazzini reported it on living Styrax. On the label of the specimen just cited, as well as of another specimen of *Uleomyces sanguineus* examined by Arnaud [20] it is indicated that the fungus is growing on leaves of Styrax. This material was collected at Sao Leopoldo, Brazil, in 1904. *Parmularia styracis* is also present on these leaves, and as already indicated, this organism rather than the Styrax leaf is considered by Arnaud to be the host of the Uleomyces. Representative illustrations of the specimen just cited, as well as of another specimen or species of Uleomyces, *U. decipiens* Syd.[21] on *Pasania glabra*, Japan, 1901, are shown in Plates 3, B and C, and 4, B and C, respectively. They illustrate the practically certain foliar origin of the conspicuous ascoma in Uleomyces. The general appearance of *U. decipiens* is shown in Plate 4, A and B, while in Plate 3, A, D, E, and F, as labeled, is shown the general appearance of fructifications of both *U. sanguineus* and *Parmularia styracis*, as well as a section through a fruit body of the latter fungus showing its attachment to the leaf.

SUMMARY

This paper contains most of the available data concerning a little-known disease of Canavalia, here termed "scab," which is said to affect *C. gladiata* and *C. ensiformis*. It is pointed out that on account of the confusion between these two species, as well as between *C. ensiformis* and *C. luzonica*, it is not definitely known at present whether *C. ensiformis* is actually susceptible to its attack. On the basis of scabbed material of Canavalia from Singapore in the United States National Herbarium, the Malay Peninsula is added to the known range of the disease. Previously this included Ceylon, the Philippines, and Java, in which country, so far as known, the disease was discovered, as well as first reported by Raciborski about 30 years ago.

Other supplementary data presented deal with the symptoms of the disease, the characteristics of its causal fungus, *Elsinoe canavaliae*, and the status of the classification of this organism.

Microconidia or other conidia representing the hitherto unreported conidial stages of *Uleomyces sanguineus* and *Myriangina mirabile* are reported, these being similar to those here noted for the first time in *Elsinoe canavaliae*, but previously seen in *Sphaceloma symphoricarpi* and *S. fawcettii*. The occurrence of the 2-layered ascus in Myriangina is reported.

[20] Sao Leopoldo, Brazil, 1904, J. Rick. (RICK, J. FUNGI AUSTRO-AMERICANI, Fasc. 3, No. 49) (*32, p. 309*), labeled *Ascomycetella sanguinea* (Speg.) Sacc.

[21] Japan, 1921, T. Yoshinga. (SYDOW. FUNGI EXOTICI EXSICCATI, Fasc. 2, No. 513) (*37*).

The data are presented for reference in connection with investigations of myriangioid fungi, but primarily because of their direct bearing on scab of Canavalia, which seems to be of some economic importance, as well as on a destructive disease of Lima bean now being investigated.

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LIMA-BEAN SCAB CAUSED BY ELSINOE[1]

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INTRODUCTION

This paper embodies the results of the writer's recent study of a destructive fungous disease which affects both bush and pole Lima bean (*Phaseolus lunatus macrocarpus* Benth.) in Cuba and Porto Rico, but which is not known to occur in the United States. The material on which the work was based consisted of diseased leaves, stems, and fruits of Lima beans, collected in November, 1929, and during the late winter and early spring of the past three years at Wajay and Hoyo Colorado, Cuba, by Prof. S. C. Bruner and Sr. Cecilio Aguiar, and also of green unshelled Lima beans intercepted at the ports of New York and New Orleans and in Porto Rico by inspectors of the United States Plant Quarantine and Control Administration. A few additional specimens representing Cuban shipments were taken from markets at Washington, D. C., by G. G. Becker, of the Plant Quarantine and Control Administration, and by the writer. One specimen taken by the Federal Horticultural Board in 1923 had been deposited in the Mycological Collections of the Bureau of Plant Industry. This was intercepted from a Cuban shipment entered at the port of New York. All the other specimens were collected between January 17 and March 30, 1930. Fully 100 specimens were intercepted at New York from Cuban shipments entered at that port; the 1 from New Orleans, also of Cuban origin, was taken from ship's stores. Of the 4 specimens from Porto Rico, 3 were from shipments about to be sent to New York, while the fourth was from the planting from which one of these shipments had come.

A similar if not the same disease affects Canavalia in Ceylon, Java, and the Philippines (*9*),[3] but even though possibly identical with it, the Lima-bean disease is here discussed separately. Most of the available data pertaining to the Canavalia disease have been published by the writer (*9*) with the explanation that this was done because of their direct-bearing on the Lima-bean disease.

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[1] Received for publication July 21, 1930; issued January, 1931. A summary of this account, amplified from a paper read before the Botanical Society of America, Mycological Section, December 30, 1929, appeared in the following publication: JENKINS, A. E. A SCAB DISEASE OF LIMA BEAN IN CUBA AND PORTO RICO. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rpt. 14: 96–97. 1930. [Mimeographed.] It was previously referred to (*8*) under the title "Lima-Bean Scab Caused by *Elsinoe canavaliae* Rac."

[2] Thanks are due Prof. S. C. Bruner, Departamento de Fitopatologia y Entomologia, Estacion Experimental Agronomica, Santiago de las Vegas, and Sr. Cecilio Aguiar, Chief Inspector, Seccion de Sanidad Vegetal, Secretaría de Agricultura, Commercio y Trabajo, Cuba, for specimens on which were based most of the studies here reported; and to E. R. Sasscer and H. S. Dean, of the Plant Quarantine and Control Administration, U. S. Department of Agriculture, for many additional specimens and for the information which is summarized in Table 1. The color readings, which are based on Ridgway,[4] as well as the color drawings, are by J. Marion Shull. Photographs and photomicrographs are by J. F. Brewer. The sections were made by means of the freezing microtome.

[3] Reference is made by number (italic) to Literature Cited, p. 22.

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C., 1912.

THE DISEASE

NAME

Cuban growers of the Lima bean refer to the disease under study as "verruga." [5] Anthracnose is the name commonly applied to diseases of similar etiology or symptoms (*8, 10, p. 46–47*). Stoneman (*19*) and Shear (*16*) have already noted that this word was designed for the familiar disease of grape which it connotes. Shear called attention to the fact that it was originated by Fabre and Dunal (*7*) in 1853 in connection with their discussion of that disease. It could thus appropriately signify the legume disease or diseases mentioned, except that, as first applied by Scribner (*15, p. 361*) in 1888, it is already in common use to denote the legume disease caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav. It was with these facts in mind, as well as because of its resemblance to citrus scab, that the writer (*9*) proposed for the Canavalia disease the name scab rather than anthracnose. With this fuller explanation, the term "scab" is here employed in referring to the Lima-bean disease.

HISTORY AND IMPORTANCE

The earliest records at hand of the observation of Lima-bean scab are those furnished by specimens of scabbed Lima bean collected in Cuba between 1914 and 1916 by J. R. Johnston and the specimen intercepted by the Federal Horticultural Board in 1923. In submitting the first specimen for diagnosis, Professor Bruner stated that the disease had been brought to his attention several years previously by Sr. Cecilio Aguiar and that Cuban plant inspectors had confused it with bean anthracnose. As to the importance of the disease, he stated that the spotting greatly disfigures the pods, which may themselves be destroyed when the tender peduncles are attacked, and that where grown for export the spotted pods are separated as culls, thereby causing considerable loss to the grower. He [6] wrote that the plantings at Hoyo Colorado, Cuba, from which specimens were forwarded in March, 1930, were more severely attacked than any he had yet observed. He stated:

> The Lima beans, pole variety, had been planted in a banana field and thus received more shade than usual. A careful count was not made, but a conservative estimate is that not less than 75 per cent of the pods were diseased. * * * It was discovered that the disease not only disfigures the pods by the unsightly spots, but that it causes many of them to wither, die, and fall off, or prevents them from filling out and developing normally. This is due to the infection of the tender stems of the young pods, producing a slow strangling effect. * * * Many half-grown pods which had been killed in this way, as well as others in a more or less withered condition * * * were observed still clinging to the vines. * * * The spots occurred quite commonly on the outer exposed side of the pod, and at times on the highest point on the plant where there are no overhanging leaves or branches.

On July 10, 1930, Professor Bruner wrote that there had been a general increase of affected plants during the current year, that practically all the commercial fields in some provinces were affected, and that the disease was the most important of those attacking the crop in Cuba.

Although severely scabbed, the green Lima-bean fruits received from the Plant Quarantine and Control Administration during the ship-

[5] Data contained in letter from Professor Bruner dated Mar. 10, 1930.
[6] Letter dated Mar. 7, 1930.

ping season just past (November, 1929, to March, 1930) were generally thrifty and well filled, and in no case were the seeds affected. Only occasional lesions of the anthracnose were seen, and comparatively few of other diseases. Upon learning that the first two samples, received on January 17 and 21 (N. Y. Nos. 13799 and 13815), were affected by scab, the New York representative of the administration reported that the disease had been more or less prevalent in all shipments from Cuba during the present importing season, and furthermore, that intercepted green Limas from Cuba, showing what appeared to be the same disease, had "in the past years * * * been diagnosed as affected by anthracnose." The specimen referred to earlier as intercepted in 1923, which was labeled *Colletotrichum lindemuthianum*, as well as other records of the Federal Horticultural Board and of the Plant Quarantine and Control Administration, had already revealed this to be the case. Some of these records have been published (*20*, *21*).

February and March, 1930, are the only months for which the Plant Quarantine and Control Administration has figures that indicate the prevalence of scab in green unshelled Lima beans entered at the port of New York from Cuba. These figures, which are incomplete for March, are presented in Table 1, which also contains the amount and distribution of green unshelled Limas from the same source during the other three months of the shipping season just past. The totals of scabbed shipments for March do not include several consignments in which scab was reported as present by the inspectors of the administration, but for which specimens were not submitted to the Washington office. Additional information shows that the number of scabbed pods in individual shipments received from Cuba or Porto Rico during the past shipping season varied from a small quantity to 100 per cent. For example, the number of infected pods in nine Cuban shipments, or 4,936 containers, received in March, ranged from 5 to 90 per cent, and in the shipments from Porto Rico, from 10 to 15 per cent. During the entire shipping season a total of 113 intercepted shipments of beans on which the disease was present were received from ports distributed as follows: 108 from Cuba through New York, 1 from Cuba through New Orleans, and 4 from Porto Rico through San Juan.

TABLE 1.—*Lima beans entered at the port of New York from Cuba from November, 1929, to March, 1930, inclusive, and prevalence of scab on February and March shipments*

Month	Shipments	Containers in shipments	Containers examined		Samples of diseased beans taken	Samples affected by scab	Shipments represented by scabbed samples	Containers in shipments from scabbed samples
	Number	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Number*	*Number*
November	69	10,016						
December	131	29,491						
January	104	10,536						
February	139	23,587	474	2	87	82	79	18,445
March	98	24,431	454	2	21	20	19	8,131

SYMPTOMS

The presence of the disease may usually be recognized by the characteristically thickened, raised, or swollen lesions first appearing on the young, growing parts of the plant. Including their histologic structure, not described in this paper, they are of the same general character and appearance as those of scab of Canavalia (*9*). The following description is based upon laboratory studies of fresh material received from Cuba and Porto Rico, supplemented by certain field observations by S. C. Bruner.

Leaf Spot

The leaf lesions are generally more pronounced on the upper leaf surface than on the lower, apparently differing in this respect from scab of Canavalia. They are often not noticeably thickened. From a few to several hundred (pl. 1, A and B) may occur on one leaf. These may be scattered over the surface of the blade or grouped along the veins. They are usually circular, ranging from minute dots to areas about 4 mm. in diameter. Those near the veins, however, are sometimes considerably larger and elongated in the direction of the veins. They may be convex on the upper surface and concave on the lower. (Pl. 2, A and D.) In color they are often a uniform vinaceous buff.[7] Certain appreciably thickened lesions, however, were liver brown in part and discolored only on the upper surface. Sometimes the leaf tissue about the spots is killed. (Pl. 1, B.) Diseased leaves may be distorted or they may become broken and torn as a result of the breaking away of affected parts. (Pl. 1, B.) Lesions on the petioles are rough and pinkish brown and may involve the entire surface or only a part.

Stem Canker

On the stems (pl. 1, C and D) the individual lesions or cankers are often somewhat raised, ranging from minute spots to elliptical or elongate areas 1 cm. or more in length, with the longer diameter parallel to the axis of the shoot. None of the individual lesions examined completely surrounded the stem, although where confluent they not uncommonly encircle it for a distance of at least 2 cm. In color the lesions on this part of the plant are generally vinaceous buff, like many of the leaf lesions. They are sometimes bordered by a purplish-brown band of varying width.

Pod Spot

The pod lesions constitute the most noticeable symptoms of the disease. Individual lesions on green fruits are irregular, elliptical, or subcircular, ranging from mere flecks to areas 5 to 8 mm. or sometimes somewhat over 1 cm. in greatest diameter. The line of the

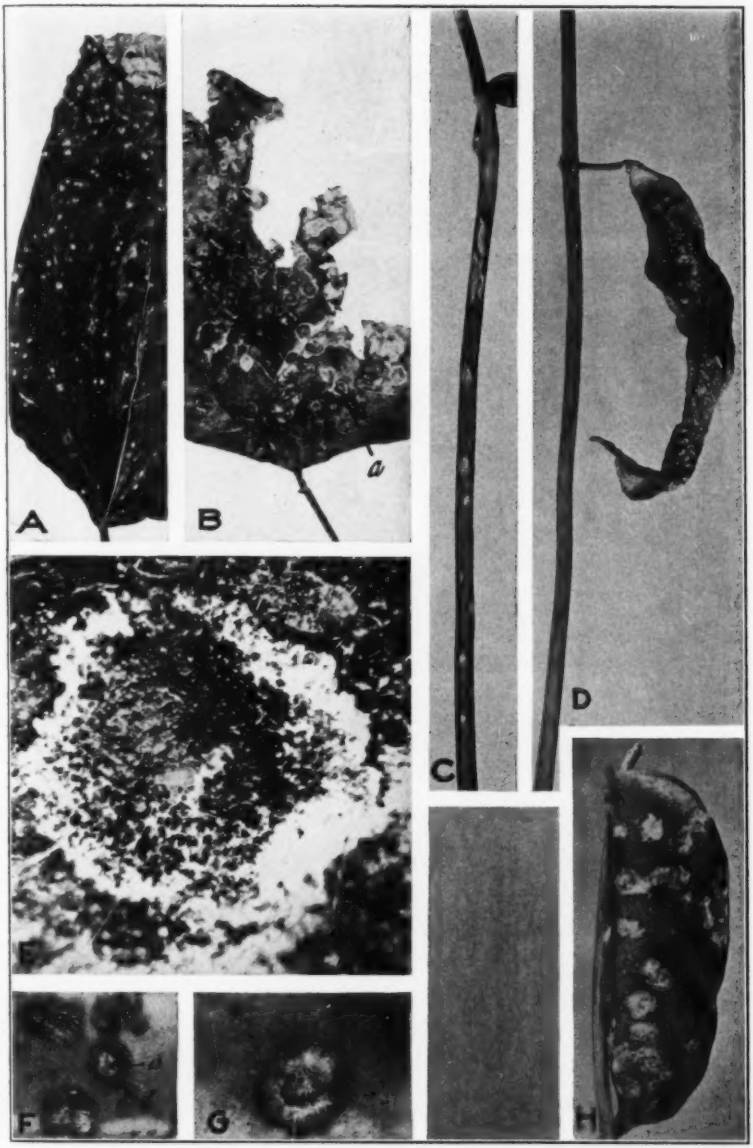
[7] Ridgway, R., op. cit.

EXPLANATORY LEGEND FOR PLATE 1

A-E.—Scab lesions on leaves (A, B, E), stems (C, D), and young fruit (D), from S. C. Bruner, Hoyo Colorado, Cuba, March, 1930. A-D, × 1; E (enlargement of B, *a*), × 23.

F, G.—Scab lesions on parts of two full-grown green fruits, from Cuba through United States Plant Quarantine and Control Administration. Light-colored central region with broad purplish border is shown in F, *a* and *b*. × 2.

H.—Scab lesions on a full-grown green fruit, from S. C. Bruner, Wajay, Cuba, late winter or early spring of 1928. × 1.



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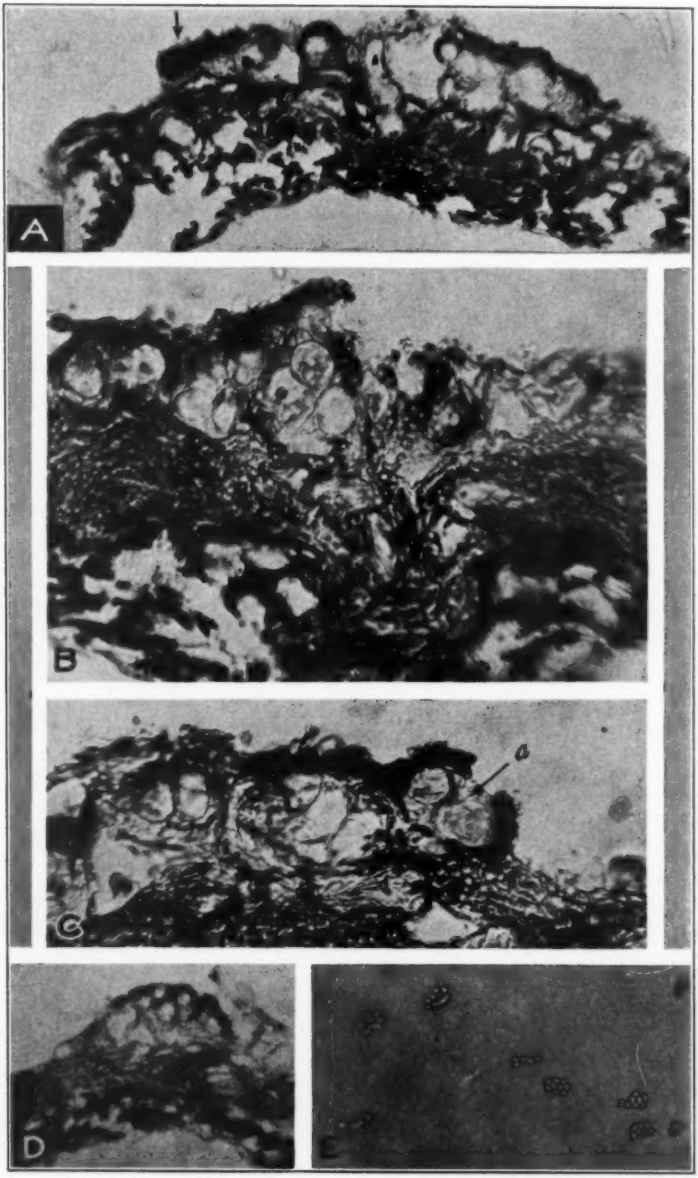

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longer axis commonly extends obliquely across the pod from the lower suture toward the apex. The lesions are often distributed irregularly over the pod surface, although not uncommonly they occur in greatest abundance on but one valve; they may be grouped toward the apex of the pod or along one or both sutures. (Pl. 3, A, and pl. 1, H.) Lesions on one valve near either suture may involve the other valve to a limited extent, but more often they do not extend across the suture. When not circular, the longer diameter of such lesions not infrequently parallels the suture. (Pl. 1, H.) Where lesions are numerous or confluent, the entire surface of the fruit, or sometimes only one valve, may be affected.

Lesions may not be noticeably raised, and occasionally they are somewhat sunken. Often, however, they assume the form of a definite swelling, sometimes depressed at the center and there checked in a stellate manner, exposing the green tissue of the pod. (Pl. 1, G.) Groups of lesions arranged along the edges of the sutures may so deform the margins of the pods that they resemble the notched edge of a saw. Even where sufficiently depressed to cause a bulging of the surface immediately beneath, none of the lesions examined had penetrated the ovary wall; in fact they have been scarcely or not at all visible on its inner face.

The most conspicuous symptom of the disease is the swollen, brightly colored lesions on the pods. (Pl. 3, A.) The color varies noticeably, not only in spots of different ages but also in those of practically the same age. For example, immature lesions often range from brick red to Kaiser brown, while others, somewhat older, are light cinnamon drab bordered with diamond brown, or dark Indian red bordered with Etruscan red. Somewhat older (pl. 1, F) or fully mature lesions (pl. 1, H) may be avellaneous to lighter at the center, with a border varying from Indian red to maroon. Confluent lesions are often variegated. Other colorations of the lesions occasionally seen are Morocco red and Napal red.

THE FUNGUS

IDENTITY, HISTORY, AND CLASSIFICATION

The fungus causing scab of Lima bean is here tentatively identified as *Elsinoe canavaliae* Rac. (*13*), the cause of scab of Canavalia. This ascomycete was discovered on the Asiatic sword or saber bean (*C. gladiata* (Jacq.) DC.) at Buitenzorg, Java, by Raciborski (*13*), who reported as well as described it in 1900, making it the type of the genus Elsinoe, now classified in the Myriangiales. The present writer (*9*) has explained that although reported in Ceylon and the Philippines on jack or horse bean (*C. ensiformis* (L.) DC.), a species "native to the West Indies and the adjacent mainland" (*12*), and very closely allied to the sword bean, it is not clear from the literature whether this is actually attacked by *Elsinoe canavaliae*. Additional data pertaining to the history and classification of the pathogene are

EXPLANATORY LEGEND FOR PLATE 2

A-D.—Sections through leaf lesions showing ascomata on upper side of lesion. A (arrow), group of conidiophores; C, *a*, asci probaly of subepidermal origin. From S. C. Bruner, Hoyo Colorado, Cuba, March, 1930. A-C, × 380; D, × 130.

E.—Conidia developed on the surface of ascospores. From Cuba through United States Plant Quarantine and Control Administration between January 17 and March, 1930. × 380.

contained in the writer's (*9*) publication dealing with scab of Canavalia. It is noteworthy that no scab was found on the jack bean growing between the rows of Lima bean from which were harvested the scabbed Lima-bean fruits shipped from San Juan, P. R. This statement is based upon the writer's examination of leaves and stems of jack bean from that field, forwarded by the inspector of the Plant Quarantine and Control Administration who discovered there the scabbed condition of the Lima beans.

Much further investigation will be required, however, before it can be definitely determined whether or not the two closely similar legume diseases here discussed are caused by the same species of Elsinoe. Very few data are available pertaining to the morphology of *E. canavaliae* on Canavalia. No fully mature or living material from this host has been available to the writer, nor have there been observed other than microconidia representing its Sphaceloma conidial stage (*9*). In so far as corresponding morphological characteristics may be compared, however, the Elsinoe on Lima bean is certainly indistinguishable from that on Canavalia. Even its orientation in the host tissues is essentially the same. Based upon the specimens that have been available for examination, there appears to be this difference in host reaction, however; in the case of Canavalia the more commonly infected leaf surface appears to be the lower, and in that of *Phaseolus lunatus macrocarpus* the upper.

The Lima bean is regarded as native to South America (*4*, *12*). It seems pertinent to note Piper's (*12*) report on the occurrence of "wild forms * * * in Cuba, Porto Rico, and Guadeloupe, from Mexico to Panama, and in Colombia, Venezuela, Brazil, and Peru, and doubtfully in Argentine." E. D. Merrill [8] states that this legume is now naturalized and is common and widely distributed in the Philippines; that it was introduced from tropical America at an early date; and that it occurs in those regions in the Philippines where sword bean, jack bean, and other species of Canavalia are grown.

In the lists that record *Elsinoe canavaliae* on sword and jack bean in the Philippines (*2*, *3*, *14*, *22*) and in Ceylon (*5*, *6*) no mention is made of this fungus on the Lima bean, although a number of other fungi are reported on that host. The taxonomic and physiologic significance of these data remains to be determined. Assuming that the Elsinoe on Lima bean and on sword bean are identical, as is done in this account, there is the possibility that they represent physiologic forms; that is, the fungus from one host may not infect the other.

MORPHOLOGY

Hyphae

Hyaline hyphae or stromata of the Elsinoe are shown in Plate 4, A, *b*, B, *a*, and C, *c*. These preparations represent sections through the outer part of young lesions on fresh pods. The first section is from a pod placed for a few days in a moist atmosphere; the other two were made on the day the material was received. The orientation of the hyphae here corresponds exactly to that described by Arnaud (*1*) for their distribution in leaf lesions on Canavalia; that is, they have grown between the phellem (pl. 4, A, *c*) and the epidermis, as well as within the disrupted epidermis and the outermost

[8] Letter dated Mar. 10, 1930.

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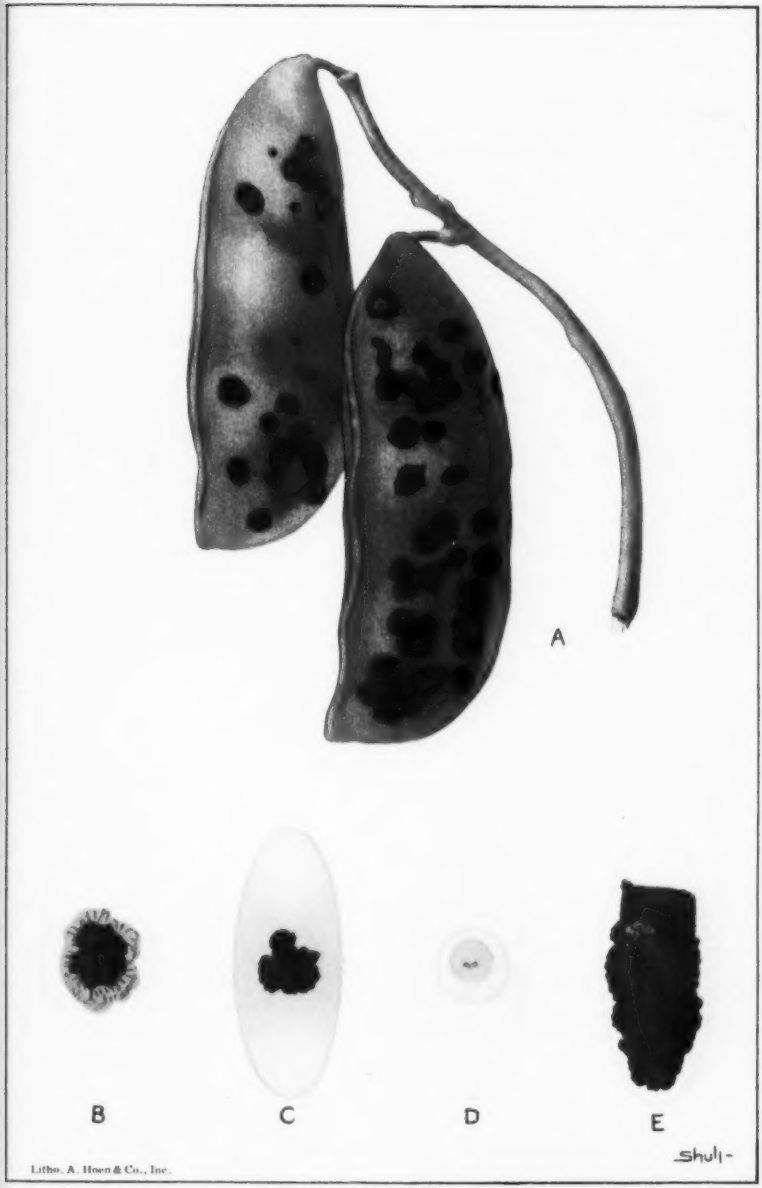


A.—Fresh Lima-bean fruits showing scab lesions. From Cuba, received through United States Plant Quarantine and Control Administration between January 17 and March, 1930. × 1.

B–E.—Four-weeks-old (B–D) and ten-weeks-old (E) cultures of *Elsinoe canavaliae* on potato-dextrose (B), beef (C), corn-meal agar (D), and potato-plug (E) media. Isolated from infected pods sent by S. C. Bruner from Wajay, Cuba, November, 1929. × 1.

broken cells of the phellem. In some instances, however, hyphae penetrate the phellem and phellogen for some distance. The walls of the subepidermal hyphae shown in Plate 4, B, *a*, have become almost completely gelatinized. Where more or less exposed the hyphae or stromata become dark. (Pl. 5, D, and K, *b*, and pl. 2, B–D.) Suggestive of Phyllachora, they are then often visible as conspicuous brownish or black coverings or patches on the surface of the lesions. (Pl. 1, E.) On account of the formation of the perfect stage of the fungus in the region immediately beneath, they may be arched, pebbled, or broken. (Pl. 5, D and K.) As explained in the following paragraph, however, the dark coloration of the fungus on the surface of the lesions may also be due to the development there of the conidial stage.

Conidial Stage

CONIDIOPHORES

The conidiophores of the Elsinoe soon become colored, as is characteristic of the form genus Sphaceloma. They generally develop from the hyaline intraepidermal hyphae in the central region of recently formed lesions (pl. 4, B, *b*, and C, *b*) or in the younger or marginal zone (pl. 5, H) of older lesions. Sometimes they form a more or less continuous dark layer over the surface of the stroma. (Pl. 2, A, arrow.) In size they range from a few to 20μ in length by about 4μ in width. They correspond to the vertical hyphae observed by Millardet (*11, p. 12, pl. 3, fig. 27*) and Starbäck (*17, p. 42, pl. 1, fig. 33*) as arising from the surface of the ascoma in *Myriangium duriaei* Mont. and Berk., and in *M. thallicolum* Starb., respectively; they were not interpreted by these authors as representing conidial fructifications.

CONIDIA

Conidia are probably readily produced from the conidiophores under favorable conditions. In the material examined, however, only a few were seen actually attached to the conidiophores. These consisted of hyaline spherical microconidia identical with those recently reported for this species on Canavalia (*9*) and ovoid or elliptical pale-colored conidia about 10μ by 4μ in size. Younger or hyaline conidia of the latter type, that is, the kind usually reported for the genus Sphaceloma, have as yet not been seen on the Lima-bean host. They were abundant, however, in young agar cultures (pl. 4, D–F), which will be referred to later in the section dealing with the cultural studies of the fungus. The elliptical 1-septate structure shown in Plate 4, A, *a*, is a swollen conidium. Although they probably occur, Coniotheciumlike conidia, such as those reported by the writer (*9*) for *Sphaceloma symphoricarpi* Barrus and Horsfall, have not as yet been seen in *Elsinoe canavaliae*.

Perfect Stage

ASCI

In the description of the genus Elsinoe, of which *Elsinoe canavaliae* is the type, it is explained that perithecia or receptacles (disci) are lacking and that the asci are generated at intervals in the subepidermal stroma. The asci are said to develop singly or in groups, sometimes with their walls in contact. On Lima bean not only are

they distributed as just described (pl. 2, B–D; pl. 4, C, *a*; pl. 5, A, D, and L), but they may also form within the epidermis. The group of asci shown in Plate 2, C, *a*, is probably subepidermal in origin. Where asci are exposed, as frequently occurs, they may form a hymenium simulating the Exoascaceae; occasionally they are present in the same pustules with the conidiophores. Where they break through the epidermis (pl. 4, C, *a*), they may simulate Magnusiella, near which Raciborski (*13*) classified his new genus Elsinoe. In some instances there were seen hyphae apparently leading up to the bases of the asci as illustrated by Arnaud (*1, pl. 10, J*) for *Atichia millardeti* Rac. In an individual group the asci may be in practically the same stage (pl. 5, A, K, *c*, and L) or in different stages of development (pls. 2, B; 4, C, *a*; and 5, D). The preparation of the fungus shown in Plate 5, A, represents an ascoma, similar to that illustrated in Plate 5, D, as dissected from the surface of a lesion, transferred to a slide, and then crushed under a cover glass.

The largest asci seen in Elsinoe on Lima bean have been somewhat larger, as well as more stipitate, than those observed in this fungus on Canavalia. Those of which the wall was still intact reached 40μ in length by 30μ in width. Here the outer inelastic primary membrane was observed to have become ruptured (pl. 5, A, *a*, E, and I) allowing the thickened inner or secondary layer to expand in the form of a clavate ostiolate structure often twice the length of the original ascus; sometimes it was entirely separated from the primary membrane. (Pl. 5, F.) The region of greatest elongation appears to be the thickened apical part as seen in the undifferentiated ascus. (Pl. 4, C, *a*, and pl. 5, D.) The double wall of the ascus in this genus of the Myriangiales has not been reported previously, nor has it been made the subject of special study by the writer. Its interpretation here as such and the terminology employed are those of Millardet (*11*) in regard to homologous structures in *Myriangium duriaei*. Stevens and Weedon (*18*) and others have presented diverse interpretations of these structures in the Myriangiales.

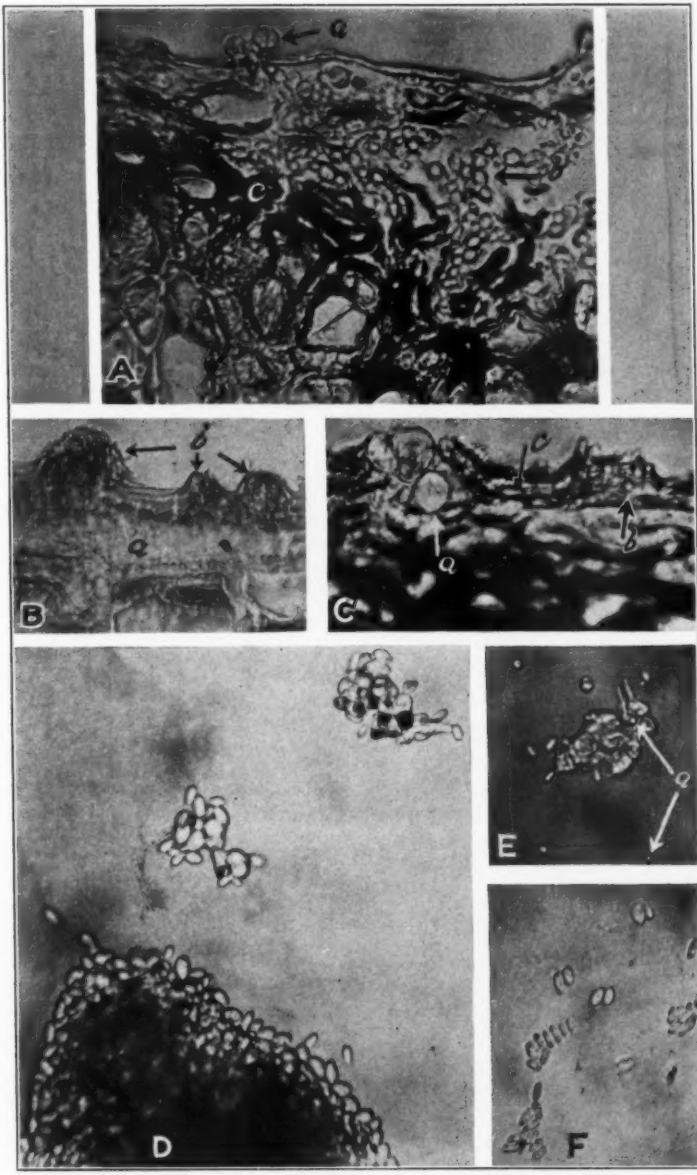
In studying the Elsinoe on Lima bean it was observed that, instead of containing ascospores, the apparently undifferentiated ascus was sometimes filled with microconidia. These are seemingly freed in a body, since in some preparations there were seen free spherical conidia in groups which still retained the shape of the ascus cavity, although larger than that space because of the swelling of the conidia.

In one instance, in which an infected pod was placed in a moist atmosphere, small yellowish masses at length appeared on the surface of the lesions. No spores or conidia were present in them. They may have been composed of the matrix in which the spores were produced in the ascus, together with the secondary layer possibly extruded in connection with the discharge of spores from the asci in situ in the lesion. The forcible ejection of spores from asci was proved by so placing parts of fresh valve lesions in the cover of an inverted

EXPLANATORY LEGEND FOR PLATE 4

A–C.—Sections through outer part of young pod lesions showing hyphae or a stromatic mass (A, *b*; B, *a*; and C, *c*); acervuli (B, *b* and C, *b*); swollen septate conidium (A, *a*); phellem layer (A, *c*); and perithecia breaking through the epidermis (C, *a*). Material from S. C. Bruner, Wajay, Cuba, November, 1929. × 380.

D–F.—Production of conidia in 2 day-old agar cultures inoculated with particles of a stromatic mass from an old agar culture; colonies in D photographed in situ without cover glass; E, *a*, refringent granules. × 380.



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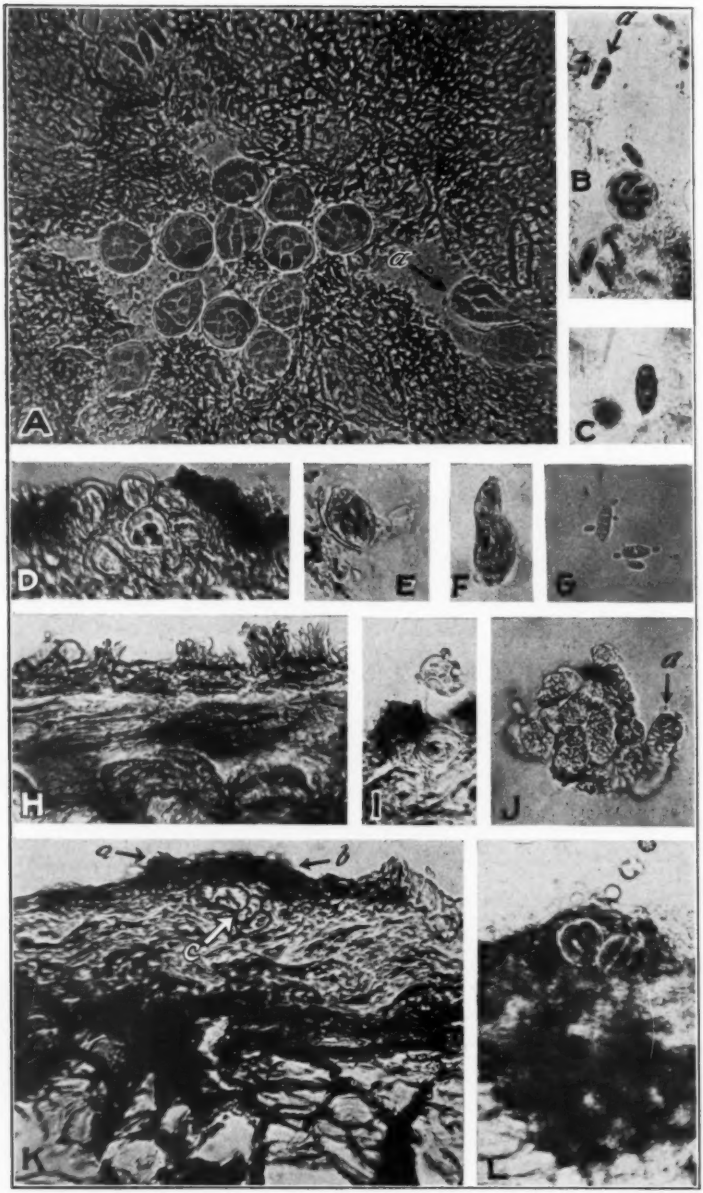

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Petri dish containing agar that the spores could be shot upward on the agar surface. The cover was lined with moist filter paper, and the inoculum elevated by placing under it one or two glass slides. In Plate 2, E, are shown eight ascospores or conidia produced from them as thus discharged on the surface of an agar plate during an 18-hour period and apparently from a single ascus.

ASCOSPORES

The brown as well as muriform ascospores (pl. 5, B, *a*, and C) not previously observed, although inferred by Arnaud (*1*) to occur in *Elsinoe canavaliae*, have been seen in this fungus on Lima bean. Their occasional red coloration may be due to staining from the coloring matter in the diseased tissues in which was borne the ascoma containing them. The ascospores, which like the conidia may become greatly swollen (pl. 5, J), commonly produce spherical (pl. 5, J, *a*) microconidia or larger ovoid or elliptical conidia. Those shown in Plates 5, G, and 2, E, represent, respectively, development in a water culture within 30 minutes and in a glycerin-agar culture within an 18-hour period. The conidia in the agar culture practically mask the asci from view. The greatly swollen spores shown in Plate 5, J, are from a pod lesion held for 24 hours in a moist chamber. These ascospores, like the eight that were ejected on the agar plate, are probably from a single ascus. The production of spherical microconidia was also observed to take place from young ascospores in situ in the ascus, as reported by the writer (*9*) for *Myriangina mirabile* (P. Henn.) Von Höhnel.

CULTURAL CHARACTERISTICS

In Plate 3, B–E, are illustrated 4-week-old slant cultures of the fungus grown on potato-dextrose, beef, and corn-meal agar media and on 10-week-old potato-plug medium. Conidia were not present in any quantity, if at all, in these test-tube cultures. Ovoid or elliptical, mostly biguttulate, conidia were abundant, however, in a 2-day-old culture made by inoculating the surface of a freshly poured corn-meal agar plate with small particles of stromatic growth from such cultures. (Pl. 4, D–F.) Conidial formation was apparently here stimulated by the transfer of the fungus to a more moist atmosphere than that in which it had formerly been growing. Although conidia were not present in old agar cultures, they were numerous on the surface of colonies or sporodochia developed from 5-day-old tissue cultures. They were then mostly swollen; some were 1-septate and developing germ tubes. These cultures were made by placing por-

EXPLANATORY LEGEND FOR PLATE 5

A.—Crushed preparation of an ascoma on surface of pod lesion. *a*, Perithecium showing outer membrane ruptured.
B–C.—Ascus and hyaline ascospores (B), a muriform ascospore (B, *a*), and a reddish-brown ascospore (C).
D.—Section through ascoma on surface of pod lesion.
E.—Ascus showing ruptured primary membrane.
F.—Expanded secondary layer of ascus.
G.—Production of conidia from ascospores in water culture.
H.—Acervuli in marginal region of pod lesion.
I.—Ascus on surface of lesion, inner part of which has separated from the thin transparent primary layer, at its base an immature ascus and at left dark stroma.
J.—Greatly swollen ascospores producing microconidia (*a*).
K.—Section through pod lesion showing acervulus (*a*) rising from the intraepidermal hyphae (*b*), beneath which is a group of young asci (*c*).
L.—Well-developed spores in situ within asci in ascoma.
Material from S. C. Bruner, Wajay, Cuba, late winter and spring of 1928 and 1929. All × 380.

tions of disinfected pod lesions on the surface of agar in a Petri dish. Isolations were made from ascospores as well as from tissue cultures. Refringent granules present in some of the hyphal cells composing the stromata in the test-tube cultures (pl. 4, E, *a*) correspond to those observed by Millardet (*11*) in *Myriangium duriaei*.

SUMMARY

A fungous disease, called scab, affects Lima bean in Cuba and Porto Rico. This disease was formerly confused with bean anthracnose. A specimen of scabbed Lima bean collected in Cuba between 1914 and 1916 constitutes the earliest available record of the disease. Although the pathogene has probably been transported many times into the United States, it is not known to be established here. Earlier record of its occurrence in Porto Rico has not been found.

In the field, leaves, stems, and fruits are affected, and when severely diseased the fruits may fail to develop. In no case in the Lima-bean fruits examined, including those of marketable size, have the lesions penetrated to the seed. This disease is of economic importance not only because of its ravages in the field but also on account of the loss in marketing due to the conspicuous disfiguration of the pods. It is the most important disease of the crop in Cuba.

The symptoms of the disease, together with the morphology and cultural characteristics of the fungus, are discussed.

The pathogene is tentatively identified as *Elsinoe canavaliae* Rac. This species has been known, heretofore, only in Java, Ceylon, the Malay Peninsula, and the Philippines, on species of Canavalia.

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FACTORS INFLUENCING THE EFFECTIVENESS OF ORGANIC MERCURY DUSTS IN PEA-SEED TREATMENT[1]

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INTRODUCTION

The results of treating pea seed with various chemical substances for the control of disease and for seed protection through the germination period have been reported by the writer.[2] The conclusions, based on experimental data obtained in the field and in the greenhouse, were as follows:

In general, the treatment of pea seed with organic mercury dusts has increased the percentage stand of plants. The amount of this increase was variable, being greater on seed of low vitality and when seed was planted under soil conditions of high-moisture content and low temperature. It appears that the treatment of pea seed with organic mercury dusts will usually increase the stand of plants sufficiently to justify their use under soil conditions that prevail at planting time in New York.

From the experiments mentioned above it was evident that there were at least two distinct phases of seed treatment to be considered: (1) The value of seed treatment in the control of seed-borne diseases, and (2) the protection of the seed from decay caused by soil-borne organisms prior to the germination of the seed and the establishment of the young seedling.

The value of seed treatment for the control of some of the seed-borne diseases of pea has been reported by the writer[3] and summarized as follows:

In these experiments the use of organic mercury dusts on seed infected with *Ascochyta pisi* did not control primary infection from diseased seed to the above-ground parts of the plant. Preliminary experiments tend to show that treatment of seed infected with *Mycosphaerella pinodes* or *Ascochyta pinodella* is of considerable benefit in reducing foot-rot injury.

Haenseler[4] did not notice any appreciable decrease in the amount of Ascochyta foot rot in Semesan-treated pea plots.

In the experiments conducted by the writer in 1926 and 1927 it was noted that various environmental factors, such as temperature and moisture content of the soil at the time of planting and immediately after planting, and the nature of the treating material greatly influenced the results obtained. Similar effects have been noted by Haenseler,[5] who showed that where conditions for germination were less favorable Semesan-treated peas gave an increase of 1.5 to 11 per

[1] Received for publication Aug. 5, 1930; issued January, 1931. This paper reports work done at the New York State Agricultural Experiment Station and at the Washington Agricultural Experiment Station. It is published with the approval of the directors of both stations as paper No. 169, College of Agriculture and Experiment Station, State College of Washington.

[2] JONES, L. K. STUDIES OF THE NATURE AND CONTROL OF BLIGHT, LEAF AND POD SPOT, AND FOOT ROT OF PEAS CAUSED BY SPECIES OF ASCOCHYTA. N. Y. State Agr. Expt. Sta. Bul. 547, 46 p., illus., 1927.

[3] JONES, L. K. Op. cit.

[4] HAENSELER, C. M. EFFECT OF ORGANIC MERCURY SEED TREATMENTS ON GERMINATION AND YIELD OF PEAS. N. J. Agr. Expt. Sta. Ann. Rpt. 48: 232-238. 1925.

[5] HAENSELER, C. M. Op. cit.

cent in stand as compared with untreated seed. Clayton [6] also obtained a better stand with several kinds of supposedly healthy vegetable seed by treating them with organic mercury compounds, particularly when the seed was planted under unfavorable conditions.

The present paper records the results of experiments made for the purpose of determining (1) the relative value of various organic mercury dusts in the treatment of healthy seed peas and (2) the effect of soil temperature and soil moisture on the effectiveness of the seed-treatment compounds.

RELATIVE EFFECTIVENESS OF SEED-TREATMENT MATERIALS

In 1928 field experiments were conducted in the vicinity of Geneva, N. Y., to compare the relative effectiveness of various organic mercury dusts and other seed-treatment materials. Special organic mercury dusts containing different percentages of chlorphenol mercury, nitrophenol mercury, and cresol mercury on carriers of talc, clay, or lime were compared to ascertain with which concentration and inert ingredient the best results would be obtained.[7] These dusts were compared with other seed-treatment materials. A list of the materials used is shown in Table 1.

Three varieties of peas—Green Admiral, Alaska, and Advancer—were used in the experiments, and three plantings of each variety were made. The plantings were made on different dates, beginning April 27 and extending to May 10. Fifty 1,000-seed lots of each variety in triplicate were measured and treated in February. The seed was treated by shaking it in a paper bag with the dust materials, which were used at the rate of 3 ounces of dust to a bushel of seed. At the time of planting, each 1,000-seed lot was placed in the hopper of a mechanical seed planter and three 30-foot rows were planted. The seeds that remained in the hopper, varying in number from 15 to 95, were counted. Thus, 905 to 985 seeds were used in each test. The planter was so adjusted that the seeds were dropped approximately 1 inch apart in the rows. Quite uniform spacing of the seed was obtained. The depth of planting was about 1½ inches.

Counts on the emergence of plants were made when the seedlings were 2 to 3 inches high. The results are recorded in Table 1. Records of rainfall and of soil moisture and temperature 2 inches below the surface were kept during the planting season and are correlated with planting dates in Figure 1.

Except in the case of Green Admiral, series A, which was planted on April 27, the soil moisture and temperature were favorable for germination of the seed. This is shown by the average emergence of the untreated seed, which varied from 49 to 70 per cent. Under these favorable conditions for germination the minimal benefit was derived from seed treatment. The Green Admiral, series A, showed a very marked reduction in stand of plants, which may be correlated with the heavy rainfall of April 28 and 29.

[6] CLAYTON, E. E. INCREASING STANDS FROM VEGETABLE SEEDS BY SEED TREATMENT. N. Y. State Agr. Expt. Sta. Bul. 554, 16 p., illus., 1928.

[7] The special organic mercury dusts were furnished by C. R. Orton of the Bayer Co. (Inc.), New York City.

TABLE 1.—*Results of field germination tests of treated and untreated healthy seed peas, Geneva, N. Y., 1928*

Test No.	Treatment and material used [a]	Percentage organic mercury as—			Solubility of mercury	Inert material	Germination of peas of different varieties planted on dates shown								
							Green Admiral			Alaska			Advancer		
		Chlorphenol	Nitrophenol	Cresol			Series A, [b] Apr. 27	Series B, May 9	Series C, May 8	Series A, May 4	Series B, May 9	Series C, May 7	Series A, May 10	Series B, May 4	Series C, May 10
							Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Control						27	55		63	66	58	46	61	52
2	do						32	53		63	68	65	42	63	53
3	do						22	52		58	68	60	42	61	38
4	Bayer dust		4		Low	Talc	50	56	56	65	70	48	51	69	59
5	Bayer 143		4		do	Clay	31	48	64	66	60	68	49	58	54
6	Bayer 124		4		High	Talc	30	57	58	65	67	59	51	67	57
7	Bayer 126		4		do	Clay	27	54	60	71	61	64	49	68	58
8	Bayer 133	4			Low	Talc	29	55	64	65	66	60	47	69	58
9	Bayer 135	4			do	Clay	33	52	70	69	69	68	48	54	61
10	Bayer 120	4			High	Talc	40	57	66	65	66	66	53	69	60
11	Bayer 107	4			do	Clay	34	49	70	68	70	55	47	66	59
12	Bayer II–6		6		Low	Talc	35	65	73	60	67	67	56	70	60
13	Control						25	52	68	64	66	60	53	61	58
14	Bayer 152		6		Low	Clay, talc	23	48	66	67	60	69	48	66	61
15	Bayer 125		6		High	Talc	24	48	63	65	66	62	54	58	61
16	Bayer 127		6		do	Clay	17	64	65	64	68	63	51	66	54
17	Bayer 153	6			Low	Clay, talc	18	49	72	74	70	63	53	62	58
18	Bayer 123	6			High	Clay	22	49	74	73	68	71	61	72	64
19	Bayer 205			3.7	do	Talc	21	47	70	68	66	64	47	63	53
20	Bayer 206			5.5	do	do	19	46	70	64	66	62	55	65	55
21	Bayer 207			7.4	do	do	24	51	71	67	64	59	51	65	55
22	Bayer 111	2	2		Low	do	26	52	75	67	64	62	56	71	58
23	Control						29	48	66	61	68	59	50	68	56
24	Bayer 112	3	3		Low	Talc	35	56	76	56	67	67	58	63	63
25	Semesan Jr			12.0	Medium	Lime	42	58	83	55	65	57	55	70	64
26	Semesan	35			High	do	46	67	84	59	65	67	55	73	69
27	Bayer 142	3	3		Medium	Clay	35	59	76	64	62	65	54	65	64
28	Bayer 110	5	5		High	do	35	58	80	70	66	65	52	64	64
29	Uspulun liquid, 0.25 per cent for 1 hour.						53	63	67	66	64	71	46	61	57
30	Uspulun	30			High		41	68	80	73	69	70	60	68	68
31	Mercuric chloride, 1/1000 for 15 minutes.						32	50	62	66	64	70	49	55	46

[a] Series A and series B were planted on the Nester farm. Series C was planted on the experiment station farm. Weather records in fig. 1 are from the Nester farm. The results obtained in each series are presented in the sequence of planting in the field. Laboratory germination: Green Admiral, 95 per cent; Alaska, 98 per cent; Advancer, 97 per cent.

[b] All dust materials were used at the rate of 3 ounces to 1 bushel of seed. They were thoroughly mixed with the seed by shaking in a paper bag.

TABLE 1.—*Results of field germination tests of treated and untreated healthy seed peas, Geneva, N. Y., 1928*—Continued

Test No.	Treatment and material used	Percentage organic mercury as—			Solubility of mercury	Inert material	Germination of peas of different varieties planted on dates shown								
		Chlorphenol	Nitrophenol	Cresol			Green Admiral			Alaska			Advancer		
							Series A, Apr. 27	Series B, May 9	Series C, May 8	Series A, May 4	Series B, May 9	Series C, May 7	Series A, May 10	Series B, May 4	Series C, May 10
							Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
32	Bayer 150	2	6		Medium	Clay	31	63	81	72	68	58	52	70	61
33	Control						28	55	74	65	67	66	45	59	57
34	Bayer 154	6	2		Medium	Clay	33	56	75	61	67	71	57	71	58
35	Bayer 101	10			High		39	55	78	68	68	64	54	70	55
36	Semesan Bel	10			Low	Clay	42	56	72	73	67	62	51	75	58
37	Bayer 216			8	Medium	Talc	33	63	67	70	62	66	56	65	51
38	Calomel, 100 per cent						45	69	67	64	69	71	52	69	53
39	Calomel, 50 per cent					Clay	35	67	75	67	71	64	55	70	53
40	Calomel, 10 per cent					do	32	62	70	62	66	55	51	67	50
41	Bayer 221			5.5	High	Talc	24	57	66	75	71	64	47	70	47
42	Grano Pax						33	62	81	65	71	70	55	75	63
43	Copper-oxychloride						31	61	69	66	69	67	50	62	58
44	Control						33	52	71	66	68	56	52	67	49
45	Cupro-Jabonite						31	57	76	65	63	74	45	40	48
46	Copper carbonate						31	64	75	56	63	72	49	59	57
47	Du Pont K. I. F.[c]						24	59	62	61	63	67	42	66	47
48	Du Pont K. I. C.[d]						33	56	65	57	63	72	52	67	45
49	Control						32	55		57	60	63		60	37
50	do						31	57		53	64	60		59	34
51	do						32			57	64				
	Average of controls						29	53	70	61	66	61	47	62	48

[c] 1.5 per cent of ethyl mercury chloride plus absorbent plus clay filler.
[d] 1.5 per cent ethyl mercury chloride plus clay filler.

The most consistently beneficial results from seed treatment were obtained with Uspulun dust or Semesan dust. It appears that organic mercury dusts containing less than 6 per cent chlorphenol mercury or nitrophenol mercury were relatively ineffective in protecting the seed from decay-producing organisms, and that dusts containing 12 per cent or more were most effective.

The tests to determine the relative value of lime, clay, and talc as carriers and the relative value of dusts of high and low solubility showed variable results. Differences obtained in these tests are in general within the range of the mathematical probable error. Dusts having higher percentages of organic mercury should have been used, since the tests show that dusts containing 6 per cent or less of

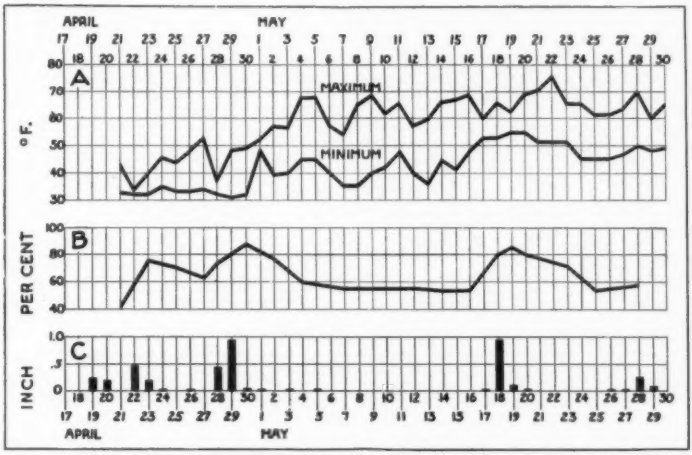

FIGURE 1.—Summary of various factors used in interpreting results of seed-treatment experiments, Geneva, N. Y., 1928: A, daily maximum and minimum soil temperatures 2 inches below the surface; B, moisture in soil as average percentage of moisture-holding capacity 2 inches below the surface; C, precipitation

organic mercury were relatively ineffective. As in previous experiments,[8] less beneficial results were obtained from treatment of seed of the Alaska variety than seed of the sweet wrinkled varieties. In the nine trials the average increase in percentage stand of plants due to treatment with Semesan dust and Uspulun dust was 10 and 11 per cent, respectively.

INFLUENCE OF SOIL MOISTURE ON EFFECTIVENESS OF SEED-TREATMENT MATERIALS

From the data appearing in Table 1 and from observations on previous similar experiments it was evident that excessive soil moisture immediately after planting is an important factor in determining the value of seed treatment. With this fact in mind, several series of experiments were conducted in the greenhouse at the agricultural experiment station, State College of Washington, Pullman, Wash., in 1928 and 1929.

[8] JONES, L. K. Op. cit.

All of the greenhouse experiments were conducted in Palouse silt loam soil brought into the greenhouse from fields where peas had never been grown. The soil had a moisture-holding capacity of 36 to 40 per cent, and prior to planting efforts were made to have the moisture content of the soil at 55 to 65 per cent of its moisture-holding capacity. In the following experiments the seeds were planted about 1 inch apart in the rows and 2 inches deep in the soil. Treated and untreated rows of each lot of seed were alternated in the benches. The seeds to be treated were shaken in a paper bag with Semesan dust, which was used at the rate of 3 ounces to a bushel of seed. In the experiments where water was added after planting, about three-fourths of an inch of water was sprinkled on the soil immediately after the seed was planted, and the soil was not again watered until the seedlings began to emerge. The temperatures at which the experiments were conducted were quite constant, fluctuating from 2 to 4 degrees, as the four sections of the greenhouse were equipped with thermostatic temperature-control apparatus.

TABLE 2.—*Effect of watering immediately after planting upon germination of Semesan-treated and untreated pea seed, Pullman, Wash., 1928* [a]

Variety and treatment	Stand of plants [b]		Variety and treatment	Stand of plants [b]	
	Soil not watered	Soil watered		Soil not watered	Soil watered
Green Admiral: [c]	*Per cent*	*Per cent*	Horsford Market Garden: [c]	*Per cent*	*Per cent*
Control	49	34	Control	67	46
Semesan treated	83	77	Semesan treated	85	77

[a] Seed was planted in 60° F. greenhouse Nov. 28, 1928.
[b] Each figure is the average of four 100-seed rows.
[c] Laboratory germination: Green Admiral, 95 per cent; Horsford Market Garden, 86 per cent.

Table 2 gives the results of the first plantings of Green Admiral and Horsford Market Garden seed obtained from seedsmen at Rigby, Idaho. In the soil watered immediately after planting the emergence of plants from untreated seed was 15 to 21 per cent less than that of plants in the unwatered soil. The treatment of the seed with Semesan was of marked benefit in each case but of greater benefit where the soil was watered after planting.

Further tests in which the same experimental procedure was used were made with 50 lots of seed produced in the vicinity of Fairfield, Wash., in 1928. The results of these tests (Table 3) are similar to those obtained in preceding experiments, and further emphasize the reduction in emergence that may occur if the soil is watered soon after planting. These trials also show the hardier character of the Alaska seed as compared with that of the sweet wrinkled varieties. With the 36 lots of Alaska seed having an average laboratory germination of 96.4 per cent there was an average decrease of 34 per cent in emergence due to watering the soil immediately after planting. In the unwatered soil an increased emergence of 14 per cent was noted from the Semesan-treated seeds. In the soil that was watered soon after planting an increased emergence of 41 per cent followed Semesan treatment of seed. The results with the sweet wrinkled

varieties were somewhat similar. Greater benefit followed treatment of the seed of these varieties in unwatered soil than was the case with the Alaska variety.

TABLE 3.—*Effect of watering immediately after planting upon germination of Semesan-treated and untreated pea seed, Pullman, Wash., 1929* [a]

Variety or type and treatment	Average laboratory germination	Stand of plants	
		Soil not watered	Soil watered
Alaska: [b]	*Per cent*	*Per cent*	*Per cent*
Control	96.4	[c] 75±1.50	41±1.88
Semesan treated		89±0.65	82±1.04
Wrinkled: [d]			
Control	88.0	44±1.88	22±2.96
Semesan treated		70±2.94	65±3.09

[a] Tests conducted in 60° F. greenhouse in January and February.
[b] Average of thirty-six 100-seed lots.
[c] Probable error of the mean, $PE_m = \frac{SD \times .6745}{\sqrt{n}}$.
[d] Average of fourteen 100-seed lots.

When it was found that watering soon after planting reduced the stand of plants, an experiment was planned to ascertain the amount of water necessary to influence germination and the effect of applying it soon after planting as compared with the effect of delaying the application for 24 or 48 hours. Three benches, 4 by 13½ feet, were each divided into five equal parts after the soil had been thoroughly mixed. A fairly uniform moisture content of 60 per cent of the moisture-holding capacity was obtained in the beds. Division of each bench was accomplished by means of wooden partitions extending from the floor of the bench to well above the surface of the soil. Fifty treated seeds and 50 untreated seeds from each of four lots were planted in each division of the three benches. The row of 50 untreated seeds of each lot was planted beside the row of 50 treated seeds from the same lot. The 15 divisions or plots were identical as to soil, soil moisture, source of seed, and plan of planting. A uniform temperature of 67° to 70° F. was maintained during the experiment. In each bench the first division received no water until the plants emerged; the second received one-fourth inch; the third, one-half inch; the fourth, three-fourths inch; and the fifth, 1 inch of water. The water was applied as uniformly as possible with a sprinkling can. In one bench the application was made immediately after planting, in another 24 hours afterwards, and in the third 48 hours afterwards. The data obtained are reported in Table 4.

With untreated seed of the Alaska variety very marked decreases in germination occurred in those plots to which one-half to 1 inch of water was applied immediately after planting, but one-fourth inch of water had no detrimental effect. It appears that the application of one-fourth to 1 inch of water 24 hours after planting had relatively little detrimental effect on germination of untreated Alaska seed and that the application of one-fourth to three-fourths inch of water 48 hours after planting increased the stand of plants from untreated seed. In these experiments the amount of water applied and the time of application appeared to have little effect on the germination of Semesan-treated Alaska seed. A slight reduction in stand from the

Semesan-treated seed was noted where 1 inch of water was added immediately after planting. In the 15 tests the average stand of plants from treated Alaska seed was 93 per cent, while that from untreated Alaska seed was 62 per cent.

TABLE 4.—*Relation of the time of water application to the effectiveness of Semesan treatment of pea seed, Pullman, Wash., November, 1929*[a]

Variety and treatment	Stand of plants					
	Watered immediately after planting		Watered 24 hours after planting		Watered 48 hours after planting	
	Control	Semesan treated	Control	Semesan treated	Control	Semesan treated
Alaska:[b]	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
No water	6½±3.31	90±0.00	66±2.21	96±0.00	66±1.93	92±1.23
¼ inch water	66±3.89	88±1.23	60±7.27	94±1.98	86±2.28	98±0.00
½ inch water	48±4.18	92±0.64	60±2.54	96±0.00	86±0.84	92±0.00
¾ inch water	32±1.92	96±0.91	56±4.69	94±0.91	76±1.23	96±1.11
1 inch water	34±2.21	80±1.79	54±1.11	94±0.97	66±4.58	92±0.84
Surprise:[d]						
No water	35	68	20	68	40	84
¼ in. water	20	42	28	54	48	82
½ in. water	20	58	12	60	26	76
¾ in. water	0	16	8	52	28	68
1 in. water	0	32	10	66	22	72

[a] Experiments conducted in greenhouse at 67°–70° F. Moisture-holding capacity of soil, 23 per cent; soil contained 60 per cent of its moisture-holding capacity; 50 seeds per row test; seed planted 2 inches deep.

[b] Each figure is the average of three 1-row tests, 1 row each of 3 different lots of seed.

[c] Probable error of the mean, $PE_m = \frac{SD \times .6745}{\sqrt{n}}$.

[d] Each figure is the percentage stand from one 1-row test.

Much better stands were obtained from the Alaska than from the Surprise seed. This difference was especially noticeable with the untreated seed and with the application of one-half to 1 inch of water even 48 hours after planting. Marked increase in stand of plants followed Semesan treatment in all the tests. The Semesan treatment was less effective, however, in protecting the Surprise seed from decay than in protecting the Alaska seed. This was especially noticeable where three-fourths to 1 inch of water was applied immediately after planting.

INFLUENCE OF SOIL TEMPERATURE ON EFFECTIVENESS OF SEED-TREATMENT MATERIALS

Further experiments were conducted to determine the effect of soil temperature on germination and on value of seed treatment. The results are reported in Table 5. These experiments were conducted at the same time as those reported in Table 2, and with seed from the same lots. The best germination of untreated seed was obtained at 60° F., with a reduction in stand of Green Admiral of 19 and 24 per cent at 45° and at 70°, respectively, and of 19 and 20 per cent in the stand of Horsford. Very marked increases in germination followed the Semesan treatment of the seed, with similar increases regardless of soil temperature. At 60° and 70° the plants emerged 6 to 8 days after planting, while at 45° they required 20 to 28 days for emergence.

TABLE 5.—*Relation of soil temperature to germination of Semesan-treated and untreated pea seed, Pullman, Wash., 1928* [a]

Variety and treatment	Stand of plants in—		
	45° greenhouse	60° greenhouse	70° greenhouse
Green Admiral: [b]	*Per cent*	*Per cent*	*Per cent*
Control	[c] 30±2.77	49±0.05	25±4.28
Semesan treated	64±1.46	83±0.84	82±1.92
Horsford Market Garden: [d]	48±1.44	67±2.31	47±3.49
Semesan treated	65±0.71	85±0.84	68±3.68

[a] Seed planted in greenhouse Nov. 28, 1928.

[b] Each figure is the average of four 100-seed rows.

[c] Probable error of the mean, $PE_m = \frac{SD \times .6745}{\sqrt{n}}$.

[d] Laboratory germination: Green Admiral, 95 per cent; Horsford Market Garden, 86 per cent.

SUMMARY

In a series of eight hundred 1,000-seed tests with various seed-treatment materials, organic mercury dusts containing at least 12 per cent of the mercury phenolate were the most effective in increasing the stand of plants. Uspulun dust and Semesan dust gave the most consistently beneficial results. Organic mercury dusts containing 6 per cent or less of chlorphenol mercury, nitrophenol mercury, or cresol mercury were of little value in increasing the stand of plants.

The experimental data presented show that the application of water to the soil soon after planting greatly reduces the percentage germination of untreated pea seed and that this reduction is greater with the sweet wrinkled varieties than with the Alaska variety. Marked reduction in stand of plants resulted from the application of one-half to 1 inch of water immediately after planting, while the application of water 24 or 48 hours after planting had little effect upon the germination of the untreated Alaska seed.

An increase in germination resulted from treating the seed with Semesan dust under various soil-moisture and soil-temperature conditions. In general, the sweet wrinkled varieties were more benefited by the treatment than the Alaska variety. With the Alaska variety, seed treatment appeared to assure a good stand of plants regardless of the moisture and temperature conditions of the soil.

The data presented in this paper indicate that the variations in soil moisture near the time of planting and differences in soil temperature cause wide variations in the results obtained from seed treatment. Such variations may account to a large extent for the contradictory statements found in the literature on the value of seed treatment.

EFFECT OF DRYING AND SULPHURING ON VITAMIN C CONTENT OF PRUNES AND APRICOTS [1]

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INTRODUCTION

The results of a study of the vitamin C content of dried fruits reported from these laboratories some months ago (*8*)[3] gave details only in regard to peaches, but, as noted in that report, similar tests with prunes and apricots, involving a larger variety of drying conditions, were also made.

So far as the writers have been able to determine, no previous study, except that of Eckman (*2*), has been reported on the antiscorbutic property of apricots fresh or dried, and only two on prunes, those of Hess and Unger (*4*) and Eckman (*2*). Hess and Unger found practically complete loss of vitamin C in dried prunes, as did Eckman in dried apricots and prunes, but in none of the tests was a comparison made with the fresh fruit, nor were any details of the drying process given. In the study of the antiscorbutic property of peaches referred to above (*8*), all of the fruit was from the same orchard, and the fresh fruit was tested along with the sundried and dehydrated products, sulphured and unsulphured. The sulphured peaches, both sundried and dehydrated, were found to have retained the full antiscorbutic value of the fresh fruit, but the unsulphured products apparently retained none. The principal questions raised by this finding were: (1) Does sulphur dioxide protect other fruits equally well? (2) If so, how much is required for such protection? and (3) How is the protective effect achieved? The present report undertakes to answer in part, at least, each of these questions.

PREPARATION OF THE FRUIT

The fruit was prepared as previously described (*8*, *9*), under the direction of members of the staff of the fruit products laboratory. Prunes of the Agen (French) variety from the 1927 and 1928 crops, grown in California, were preserved by pitting and grinding, packing in 8-ounce tin cans, and freezing solid. The frozen fruit was stored at −17° C. until fed to the test animals. It is assumed that the vitamin C content of fruit preserved in this way is not changed even after long storage. Delf (*1*) found that frozen orange juice retained its antiscorbutic efficiency after one year in storage, and preliminary tests in this laboratory with frozen orange juice of several types confirm this finding.

[1] Received for publication Aug. 25, 1931; issued, January 1930. Contribution from the laboratory of household science and the fruit products laboratory, College of Agriculture, University of California.

[2] The writers are indebted to the Dried Fruit Association of California for much of the fruit used in this study and for moisture and sulphur dioxide determinations made by R. S. Hiltner and B. F. Hatherell.

[3] Reference is made by number (italic) to Literature Cited, p. 45.

TABLE 1.—*Preparation and composition of fruits tested*

Fruit and crop	Lot No.	Method of preparation	Moisture	Net shrinkage	pH	Sulphur dioxide content
			Per cent	*Per cent*		*Parts per million*
Agen prunes, 1927 crop.	10a	Pitted, ground, frozen, and kept at −17° C.	79.5	1.0		
	11a	Dehydrated whole at 72° C. for 30 hours after lye dipping, washing, and sulphuring overnight.	11.7	4.0	3.4	887
	12a	Same as for 11a, but without sulphuring	12.9	3.9		
	18a	Lye dipped, washed, sundried 7 days, held in stack 7 days.	14.3	3.9	3.3	
Agen prunes, 1928 crop.	10	Same as for 10a	62.6	1.0	3.5	
	11	Same as for 11a	[a] 19.0	2.1	3.2	1,980
	12	Same as for 12a	18.2	2.2	3.5	
	13	Same as for 11a, but without lye dipping	18.6	2.2	3.2	1,020
	14	Sulphured overnight, whole, sundried 7 days, held in stack 7 days.	19.8	2.1	3.1	1,005
	15	Same as for 11a, but without lye dipping or sulphuring.	21.4	2.1	3.3	
	16	Same as for 14, but without lye dipping or sulphuring.	20.2	2.1	3.6	
	17	Same as for 18a, but sulphured overnight	17.8	2.2	2.6	2,695
	18	Same as for 18a	17.0	2.2	3.2	
	19	Same as for 18a, but held at room temperature.	17.4	2.2		
Royal apricots, 1928 crop.	5	Pitted, ground, frozen, and kept at 17° C.	82.9	1.0	3.5	
Royal apricots, 1929 crop.	5a	Pitted, ground, evacuated, released with nitrogen, sealed cold, frozen, and kept at −17° C.	85.7	1.0	3.7	
	1a	Dehydrated for 17 hours at 65° C.	12.8	6.1	3.6	
	2a	Same as for 1a, but previously sulphured 1 hour.	15.6	5.9	3.7	892
	3a	Same as for 1a, but previously sulphured 6 hours.	13.8	6.0	3.5	2,028
Royal apricots, 1928 crop.	1	Cut, pitted, sulphured 3½ hours, dehydrated 24 hours at 72° C.	18.8	4.7	2.4	515
	2	Same as 1, but sulphured 30 minutes	17.0	4.8	2.9	125
	3	Same as 1, but unsulphured	22.6	4.5	3.2	100
	4	Same as 1, steamed 3½ minutes, and sulphured 20 minutes.	16.3	4.9	3.2	80
	6	Cut, pitted, sulphured 2½ hours, sundried 11 days.	19.0	4.7	3.3	700
	7	Same as 6, but sulphured 30 minutes	17.5	4.8	3.1	470

[a] Flesh.

The dehydrated and sundried prunes were prepared as indicated in Table 1, and as previously described. The effect of dipping the fruit in lye was of particular interest in this inquiry, and several preparations were made with and without this treatment. All dried products were stored at 0° C.

Royal apricots of the 1928 and 1929 crops were similarly prepared, and, as shown in Table 1, an attempt was made to obtain products of different sulphur dioxide content. The frozen fresh apricots of the 1928 crop were found to have lost practically all antiscorbutic value (Table 5), and at the same time on thawing they were observed to evolve a good deal of gas, presumably air and carbon dioxide retained in the fresh tissue. Accordingly, the experiment was repeated in 1929 with similar fresh apricots, but precautions were taken to exclude air. The fruit was pitted, ground, and packed in a large jar which was evacuated and the vacuum released with nitrogen. The pulp was then packed quickly in 8-ounce tin cans, the lids given the first crimp, evacuated, and the vacuum again released with nitrogen, after which the usual freezing and storage procedure was carried out. Excellent preservation of vitamin C resulted in this product. (Table 5.) It was interesting to find this exception to the effectiveness of the ordinary freezing process. If the sharp

freezing of fruits should assume importance as a means of preservation, attention should be directed to this danger and suitable evacuation provided for fruits, such as apricots, in which retained gases are likely to promote the destruction of vitamin C.

The experience of Kohman and Eddy (*7*) with the respiratory oxygen of canned apples appears to have been similar to that of the writers with frozen apricots. However, preliminary experiments in this laboratory with canned apricots do not indicate the persistence of oxygen retention during processing.

A similar destruction of vitamin A was not observed in unevacuated frozen apricots used in a previous study (*9*). An evacuated lot (LS) from the same crop yielded no higher values for vitamin A than did the unevacuated. Apparently vitamin A is less susceptible to oxidation than is vitamin C, or the apricots do not contain as potent specific catalysts for destruction of vitamin A as for vitamin C.

The moisture, hydrogen-ion concentration, and sulphur dioxide content of the fruit samples were determined by the methods previously described (*9*, *footnote 1*). It should be noted that samples 2, 3, and 4 of the apricots shown in Table 1 were essentially unsulphured, so far as retained sulphur dioxide is concerned. Unsulphured fruit usually has an apparent sulphur dioxide content of 50 to 100 parts per million. Differences in sulphur dioxide content found by a single method within this range are therefore probably not significant.

METHODS OF TESTING FOR VITAMIN C

A modification of the diet proposed by Sherman, LaMer, and Campbell (*12*), as previously described (*8*), was used in all these tests. The basal diet contained rolled oats, 69 per cent; baked skim-milk powder, 30 per cent; and sodium chloride, 1 per cent. In addition, 2 c. c. of cod-liver oil was fed daily to each animal separately. The enlargement of rib joints in apparently nonscorbutic guinea pigs mentioned by Schwartze, Murphy, and Hann (*11*) may well have been caused by the lack of antirachitic vitamin in the basal diet which they used. It was to avoid this danger that cod-liver oil was substituted for butterfat, which was originally included. The animals were kept on the basal diet with tomato juice for several days, or until they showed a definite and rapid increase in weight, before the fruit doses were fed.

The record of the large number of negative controls accumulated over a period of seven years of continual vitamin C testing leaves little doubt as to the scurvy-producing character of this diet. Moreover, long-continued feeding of positive controls on this diet, with a daily supplement of 12 c. c. of canned tomato juice, together with the reproduction records and the rearing of second generation animals, makes the writers equally confident that the diet is complete except for the antiscorbutic factor.

TABLE 2.—*Comparison of condition of guinea pigs after 60 and 90 days feeding of fresh and dried apricots and prunes*

Fruit and lot No.	Amount fed daily	Animals	Average weight			Average gain or loss per day—		Final condition
			Initial	After 60 days	After 90 days	In 60 days	In 90 days	
	Grams	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	
Dehydrated sulphured apricot, No. 1.	3	2	347	345	246	0	−1.1	Mild scurvy.
	4	5	329	496	543	2.8	2.4	No scurvy.
	6	2	364	587	649	3.7	3.2	Do.
Sundried sulphured apricot, No. 6.	6	4	340	448	526	1.6	2.0	1, mild scurvy; 3, normal.
	8	2	356	408	355	.9	0	1, mild scurvy; 1, normal.
Fresh frozen prune, No. 10.	10	1	324	376	474	.9	1.7	Mild scurvy.
	12	4	328	377	394	.8	.7	3, mild scurvy; 1, normal.
	15	5	354	356	387	0	.4	3, mild scurvy; 2, normal.
	20	2	359	472	454	1.9	1	No scurvy.
Dehydrated sulphured prune, No. 11.	5	4	338	494	542	2.6	2.3	Do.
	8	3	326	484	555	2.6	2.5	Do.
Sundried sulphured prune, No. 17.	5	4	333	391	434	.9	1.1	3, no scurvy; 1, mild scurvy.
	8	3	323	492	422	2.8	1.1	2, no scurvy; 1, mild scurvy.

In the earlier series of experiments, including all tests with fruit of the 1927 and 1928 crops, the 90-day test period was used. At the end of the 90 days autopsy was performed on all animals and careful examination was made for the usual pathological lesions of scurvy. It was observed, however, that there was little change in the condition of even border-line cases after the sixtieth day, and, as shown in Table 2, there was little change even in the rate of growth during the first 60 days as compared with that of the entire 90-day period. Eighty seven per cent of all the animals which were only partly protected from scurvy by the smaller fruit doses, and which died before the 90-day period was over, died previous to the sixtieth day. (Table 3.) Most of the remaining 13 per cent had shown definite symptoms of scurvy by the sixtieth day. There seemed little likelihood, therefore, that accuracy would be sacrificed by reducing the period of test feeding to 60 days. Accordingly, this was done in testing the 1929 apricot preparations.

TABLE 3.—*Survival period of scorbutic guinea pigs when fed insufficient doses of fresh frozen and dried prunes, and fresh frozen and dried apricots, as antiscorbutics*

Fruit	Animals showing scurvy, due to insufficient dosage	Animals dying previous to sixtieth day		Animals dying previous to ninetieth day and after sixtieth day		Average survival period
	Number	*Number*	*Per cent*	*Number*	*Per cent*	*Days*
Prunes, 1927 crop (all products)	16	13	81	3	19	46
Prunes, 1928 crop (all products)	42	37	88	5	12	38
Apricots, 1928 crop, fresh frozen (not evacuated)	28	24	86	4	14	46
Dried apricots, 1928 and 1929 crops	41	36	88	5	12	39
Total	127	110	87	17	13	41
Negative controls	12	12	100			29

TABLE 4.—*Vitamin C content of fresh and dried sulphured and unsulphured prunes*

Treatment and crop	Lot No.	SO_2	Amount fed daily: Dried fruit	Amount fed daily: Equivalent in fresh fruit	Animals	Average weight of animals: Initial	Average weight of animals: Maximum	Average weight of animals: Final	Gain or loss in weights per day	Average length of period	Scurvy score	Final condition
		P. p. m.	*Grams*	*Grams*	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Days*		
Fresh frozen, 1927 crop	10a		5.0	5.0	2	336	425	280	−0.6	90	2	Mild scurvy.
			8.0	8.0	2	319	391	273	−1.0	47	2	Do.
			10.0	10.0	5	351	481	419	.9	78	2	2, no scurvy.
			12.0	12.0	2	322	559	515	2.1	90	0	No scurvy.
Fresh frozen, 1928 crop	10		10.0	10.0	1	324	474	474	1.7	90	2	Mild scurvy.
			12.0	12.0	4	328	434	394	.7	90	5	3, scurvy; 2, normal.
			15.0	15.0	5	354	523	436	1.0	80	8	2, scurvy; 3, normal.
			20.0	20.0	2	359	497	454	1.1	89	0	No scurvy.
Dehydrated, sulphured, lye dipped, 1927 crop.	11a		4.0	16.5	2	320	392	299	−.3	83	4	Mild scurvy.
			5.0	20.6	3	326	473	388	.7	90	2	2, mild scurvy; 1, normal.
Dehydrated, sulphured, lye dipped, 1928 crop.	11	1,980	4.0	8.7	3	311	462	368	.9	65	8	2, scurvy; 1, normal.
			5.0	10.8	3	326	518	518	2.1	90	1	No scurvy.
			8.0	17.4	3	326	561	561	2.6	90	0	Do.
Dehydrated, unsulphured, lye dipped, 1927 crop.	12a		2.0	7.9	2	325	404	244	−2.3	35	16	Severe scurvy.
			5.0	19.8	2	340	412	207	−3.2	41	14	Do.
Dehydrated, unsulphured, lye dipped, 1928 crop.	12		10.0	39.6	2	322	382	320	−.1	37	15	Do.
			10.0	21.9	2	350	380	234	−3.3	35	15	Do.
			15.0	32.8	2	315	327	240	−2.8	27	17	Do.
Dehydrated, sulphured, not lye dipped, 1928 crop.	13	1,020	4.0	8.7	2	332	356	244	−2.6	34	11	Do.
			5.0	10.9	2	348	382	265	−2.0	41	13	Do.
			8.0	17.4	2	339	430	356	.3	62	8	1, scurvy; 1, normal.
			15.0	32.7	2	342	461	334	−.1	65	8	Do.
Dehydrated, unsulphured, not lye dipped, 1928 crop.	15		8.0	16.8	2	352	389	211	−5.2	27	19	Severe scurvy.
			15.0	31.5	3	390	400	317	−2.9	25	12	Do.
Sundried, unsulphured, lye dipped, 1927 crop.	18a		5.0	19.5	2	314	357	212	−3.2	32	16	Do.
			10.0	39.0	2	339	410	247	−2.2	42	18	Do.
Sundried, unsulphured, lye dipped, 1928 crop.	18		10.0	22.2	2	319	342	231	−2.8	31	17	Do.
			15.0	33.3	2	325	347	224	−3.6	28	13	Do.
Sundried, sulphured, not lye dipped, 1928 crop.	14	1,005	4.0	8.6	2	355	381	242	−3.9	29	18	Do.
			5.0	10.7	2	373	415	267	−2.8	38	16	Do.
			8.0	17.2	1	304	356	244	−2.3	26	14	Do.
			15.0	32.2	3	365	425	246	−3.0	40	14	Do.
Sundried, sulphured, lye dipped, 1928 crop.	17	2,695	5.0	11.0	4	336	463	434	1.3	78	11	1 mild scurvy; 3, normal.
			8.0	17.6	3	323	502	422	1.2	86	2	1, mild scurvy; 2, normal.
Sundried, unsulphured, not lye dipped, 1928 crop.	16		8.0	17.0	2	329	349	252	−2.8	28	12	Scurvy.
			15.0	31.9	2	333	438	272	−2.0	31	14	Do.
Same as 18, kept at room temperature	19		20.0	44.4	2	354	413	255	−2.5	39	16	Do.

Zilva (*3, 14, 15*) in a long series of vitamin C studies used chiefly the 60-day period, and Sherman, LaMer, and Campbell (*12*) in their original communication report 70 to 90 days as the feeding period. Since the daily growth of the animals on the test doses was practically the same during the 60-day period (Table 3) as during the 90-day period, and since even incipient scurvy manifests itself before the sixtieth day, there can be little reason for carrying the test longer than 60 days even for the best quantitative comparisons possible with the biological method.

Another precaution has, however, been introduced in the histological examination of the teeth of all test animals, following Höjer's suggestion (*5*). Some guinea pigs on each of the 1929 apricot doses were killed after 14 days and their teeth examined for destruction of dentine and changes in odontoblasts. Animals on the same doses were killed after 60 days and similar examinations of their teeth were made. The results of these studies are not, however, reported here.

The "scurvy score," often mentioned by investigators of the vitamin C content of foods, has been included in all autopsy reports referred to in this paper. It was first proposed by Holst and Fröhlich (*6*), and consists of a more or less arbitrary judgment as to the degree of severity of pathological lesions in teeth, ribs, jaw, intestine, joints, and muscles. Three points indicating the most advanced stages of these lesions—fragility, looseness, or hemorrhage—in each of the eight types of tissue constitute the highest possible scurvy score, which is 24. After much experience the operator may achieve definite skill in allotting the "scurvy score," but at best the method must be looked upon as auxiliary to the use of graded doses in establishing nice distinctions among antiscorbutic minima.

EXPERIMENTAL RESULTS

PRUNES

Only the sulphured prunes and apricots retained any part of the antiscorbutic property of the fresh fruit, as had previously been found with peaches. Table 4 shows that of the 14 prune preparations, 6 were somewhat effective in reasonably small doses, 2 of these being frozen fresh fruit (lots 10, 10a), 2 dehydrated sulphured and lye dipped (lots 11, 11a), 1 dehydrated and sulphured but not lye dipped (lot 13), and one sundried, sulphured, and lye dipped (lot 17). The effect of the lye dipping was distinctly favorable to the retention of the vitamin, probably because the surface of the lye-dipped prunes was more easily penetrated by the protecting sulphur dioxide. This supposition is corroborated by the considerably higher sulphur dioxide content of the lye-dipped fruit (lots 11 and 17) as compared with the undipped fruit (lots 13 and 14). The one undipped sample of dehydrated sulphured prunes (lot 13) which retained any trace of antiscorbutic property was distinctly lower in value than the corresponding lye-dipped product (lot 11), but higher than the sundried sample (lot 14) receiving similar treatment. In all cases the sundried samples were lower in antiscorbutic value than the dehydrated ones, even when the amount of sulphur dioxide present was larger in the former.

Some question arises as to the effect of storage upon the sulphur dioxide content of the fruit. Sulphur dioxide in dried fruit disappears during storage (*10*), although rather slowly. Some discrepancy would doubtless exist, therefore, between the figures given here and the content when drying was completed or when feeding was done. However, the difference would probably be relatively small, since the determinations were made as soon as possible after the samples were placed in storage at 0° C. It is obvious that the protection afforded by the sulphur dioxide is exerted during the actual drying period and that loss of the gas later may be of little importance although not negligible, particularly if the fruit is subjected to higher than usual room temperatures. The resulphuring of dried fruit after washing and before packing, regularly practiced with apricots and peaches and often with apples and pears, can add nothing to vitamin C protection.[4]

Attention is directed to the difference in antiscorbutic property shown by the fresh prunes of the two crops. The 1927 crop with a moisture content of 79.5 per cent showed a distinctly higher vitamin C value than the 1928 crop with moisture content of 62.6 per cent. It is possibly not surprising that the juicier fruit should be richer in the water-soluble antiscorbutic substances. It was found, incidentally, that the vitamin A content of the two fresh prune samples differed in the opposite direction, the more watery 1927 lot being poorer in the vitamin. Again, the fat-soluble vitamin might well be expected to reside in the pulp and to vary with dry content. The fallacy of expressing vitamin C content in terms of dry matter, therefore, is illustrated by the contrast in the values for the fresh prunes shown in Table 4.

APRICOTS

The tendency to loss of vitamin C in frozen, sundried, and dehydrated apricots appears to be greater than in peaches or prunes. This tendency to loss of vitamin is even more marked in the case of vitamin A, and points to the probable presence of powerful oxidative catalysts as well as of tissue oxygen in apricots. In comparing the 1928 apricot samples with fresh fruit it was necessary to use frozen fruit of the 1929 crop because of the complete loss of vitamin C in the unevacuated frozen samples of the 1928 crop. The percentage losses given in Table 6 are therefore of only relative value. If the same condition shown by the two prune crops holds for the apricots, however, the more watery 1929 apricots (moisture 85.7 per cent) were slightly richer in vitamin C than the 1928 fruit (moisture 82.9 per cent), and the losses indicated for the dried preparations of the latter crop are probably somewhat too high.

[4] A preliminary abstract of a report given at the Cincinnati meeting, 1930, of the American Chemical Society by E. M. Nelson and D. Breese Jones indicates that processing and resulphuring of apricots as generally practiced results in retention of very little of the antiscorbutic content of the fruit. However, confirmation of the striking preservative effect upon the vitamin of the sulphur dioxide used in the original drying is reported. Tests upon the vitamin C content of resulphured and processed apricots of the 1930 crop are now being made in these laboratories, as well as tests of the effect of cooking upon the same vitamin in the sulphured dried apricots.

TABLE 5.—*Vitamin C content of fresh and dried sulphured and unsulphured Royal apricots*

Treatment and crop	Lot No.	SO_2	Amount fed daily		Animals	Average weight of animals			Gain or loss in weight per day	Average length of period	Scurvy score	Final condition
			Dried fruit	Equivalent in fresh fruit		Initial	Maximum	Final				
		P. p. m.	*Grams*	*Grams*	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Days*		
Fresh frozen, not evacuated, 1928 crop	5		3.0	3.0	2	355	458	225	−3.5	37	14	Severe scurvy.
			5.0	5.0	4	320	399	247	−1.8	40	11	Do.
			8.0	8.0	6	315	374	233	−1.8	45	14	Do.
			10.0	10.0	4	315	366	232	−1.8	46	14	Do.
			15.0	15.0	4	306	381	268	−.8	45	10	Do.
			20–30	20–30	3	351	382	263	−1.2	71	16	Do.
Fresh frozen, evacuated before freezing, 1929 crop	5a		15.0	15.0	3	347	476	459	2.2	51	0	No scurvy.
			20.0	20.0	3	324	466	452	2.0	64	0	Do.
Dehydrated, unsulphured, 1928 crop	3	100	1.0	4.5	2	306	336	238	−1.9	36	20	Severe scurvy.
			2.0	9.0	2	306	345	188	−3.4	35	22	Do.
			5.0	22.6	2	311	361	226	−2.7	31	18	Do.
			10.0	45.3	2	373	430	269	−3.7	28	19	Do.
Dehydrated, unsulphured, 1929 crop	1a		15.0	91.5	3	353	361	227	−3.8	33	13	Do.
Dehydrated, sulphured, steamed, 1928 crop	4	80	6.0	29.3	2	359	401	239	−3.3	36	16	Do.
			15.0	73.3	2	350	390	265	−2.1	40	16	Do.
Dehydrated, sulphured, 1928 crop	2	125	1.0	4.7	4	314	347	202	−3.9	29	15	Do.
			4.0	19.4	2	321	336	205	−3.2	36	14	Do.
			6.0	29.1	2	352	380	233	−3.1	38	18	Do.
			15.0	72.5	3	307	343	200	−3.5	31	15	Do.
Do	1	515	3.0	14.2	3	345	407	231	−1.5	74	12	Scurvy.
			4.0	17.0	5	329	546	543	2.4	90	0	No scurvy.
			6.0	28.5	2	364	649	649	3.2	90	0	Do.
Dehydrated, sulphured, 1929 crop	2a	892	5.0	29.5	4	350	533	495	2.5	58	0	Do.
Do	3a	2,028	2.0	12.0	1	381	437	230	−4.0	38	12	Scurvy.
			4.0	24.0	3	357	517	517	2.8	58	0	No scurvy.
Sundried, sulphured, 1928 crop	7	470	4.0	19.4	1	310	339	250	−1.4	42	18	Severe scurvy.
			5.0	29.8	2	370	399	258	−3.6	31	16	Do.
			10.0	48.2	4	325	418	278	−.8	60	10	Moderate scurvy.
Do	6	700	4.0	18.9	1	350	376	260	−3.0	30	12	Scurvy.
			6.0	28.4	4	340	500	470	1.5	88	4	3, no scurvy.
			8.0	37.8	2	356	428	355	0	90	1	1, no scurvy.

An attempt was made with the apricots to find the lower limit of sulphur-dioxide content which would protect the vitamin from destruction during drying. Of the 11 apricot preparations studied (Table 5), 2 were fresh frozen, 2 were sundried, and 7 were dehydrated. Only 2 of the dehydrated samples were unsulphured (lots 3 and 1a), and these were found to be devoid of antiscorbutic value. Of the 5 sulphured dehydrated preparations, 2 were of very low sulphur-dioxide content (lots 2 and 4), and these appeared to be no more valuable than the unsulphured lots. The 3 lots containing larger amounts of sulphur dioxide (lot 1, 515 p. p. m.; lot 2a, 892 p. p. m.; lot 3a, 2,028 p. p. m.) retained their antiscorbutic property satisfactorily. Of the 2 sundried sulphured specimens, lot 6 (700 p. p. m.) compared well with the best of the dehydrated (lot 1), but lot 7 (470 p. p. m.) gave evidence of very low yet demonstrable retention of the vitamin. This figure (450 to 500 p. p. m.) has therefore been taken as representing the border line of effective protection for vitamin C. Lots 2 and 4 had no more sulphur dioxide than did the unsulphured product which represented the blank check on the method of determination of sulphur dioxide, and may therefore be looked upon as practically identical with unsulphured fruit. It must be admitted also that there is disagreement as to the reliability of methods of determining sulphur dioxide in fruit, and that consequently fine distinctions can hardly be drawn at present on this point. However, the vitamin C values of these apricot samples accord remarkably well with the theory that the sulphur-dioxide content must reach a certain minimum in order to be effective in protecting the vitamin, and likewise that sulphuring beyond this point usually adds little to the protection. (Compare lots 2a and 3a.) However, the difference in vitamin retention between prune lots 11 (1,980 p. p. m.) and 11a (887 p. p. m.) may be cited as indicating some effectiveness beyond the minimum.

A recent report by Williams and Corran (*13*) indicates the failure of potassium metabisulphite as preservative of vitamin C of lemon juice but success in the case of lemon juice kept in a sulphured cask. These results seem to indicate an injurious action by one of the substances, other than sulphur dioxide, introduced in the gradual decomposition of the metabisulphite, or else an insufficient release of sulphur dioxide. Some means of adding the sulphur dioxide to fruit juices, other than by use of metabisulphites, seems indicated.

In comparing the percentage retention of vitamin C in the fresh fruit with that in the various dried products as shown in Table 6, the same assumptions were made as in the study of vitamin A previously reported (*9*). The minimum protective dose of the fresh fruit was used as the basis and was compared with the minimum protective dose of the dried product, calculated in equivalent of fresh fruit. The calculation is arbitrary and must be recognized as affording only approximately accurate comparisons.

TABLE 6.—*Summary of experimental results on vitamin C retention in dried peaches, prunes, and apricots, sulphured and unsulphured*

Fruit	Method of preparation and crop	Lot No.	Minimum daily dose required to protect standard guinea pig against scurvy (equivalent in fresh fruit)	Average daily gain or loss in weight of animals	Vitamin C retention
			Grams	*Grams*	*Per cent*
Peaches (8)	Fresh, frozen	F	5–8	1.1	100
	Dehydrated, sulphured	SE	4	1.7	100
	Sundried, sulphured	SD	8	.8	100
	All unsulphured	D, E	[a] 40+	[b]	0
Prunes	Fresh, frozen, 1927 crop	10a	12	2.1	100
	Fresh, frozen, 1928 crop	10	10–20	1.0	100
	Dehydrated and sundried, sulphured, lye dipped, 1928 crop.	11, 17	11–18	1.6	100
	Dehydrated, sulphured, lye dipped, 1927 crop.	11a	20	.7	60
	Dehydrated, sulphured, not lye dipped, 1928 crop.	13	33+	—.1	[c] 50–
	Sundried, sulphured, not lye dipped, 1928 crop.	14	32+	[b]	0
	All unsulphured, 1927 and 1928 crops	12, 12a, 15, 18, 18a, 19, 16.	44+	[b]	0
Apricots	Fresh, frozen, not evacuated, 1928 crop	5	30+	[b]	0
	Fresh frozen, evacuated, 1929 crop	5a	15	2.2	100
	Dehydrated and sundried, more than 470 p. p. m. SO_2, 1928 and 1929 crops.	1	17	2.4	[d] 100
		2a	24	2.5	62
		3a	29	2.7	52
		6	28	1.5	[d] 52
		7	38+	[e] 1.0 / [f] 1.0	39–
	Dehydrated and sundried, less than 470 p. p. m. SO_2, 1928 and 1929 crops.	1a	81+	[b]	0
		2	72+	[b]	0
		3	45+	[b]	0
		4	73+	[b]	0

[a] + indicates that more than the weight given, and which was tried, would be necessary for protection.
[b] Severe scurvy.
[c] − indicates that less than the percentage given was retained.
[d] All products dried in 1928 compared on basis of 1929 fresh apricot vitamin content; these two results are approximate.
[e] Mild scurvy.
[f] Normal.

SUMMARY

The vitamin C content of frozen fresh prunes and apricots and of prunes and apricots dried by various methods was determined by biological technic.

Doses of the different fruit products were fed to guinea pigs for 60 and 90 days, and the rate of growth of the animals receiving the various doses was compared. Examinations were also made for symptoms of scurvy, and in cases where animals died because of insufficient protection from scurvy the length of the survival period was determined. The 60-day period is shown to be as effective for assay of vitamin C as the 90-day period.

Frozen fresh prunes of two crops retained the vitamin C satisfactorily, but frozen fresh apricots packed in cases which were not evacuated, lost all of this property. A second lot packed in cases which were evacuated and filled with nitrogen before the fruit was frozen, retained the vitamin. The difference is ascribed to retention of tissue respiratory oxygen in the unevacuated lot.

Sulphured, dehydrated, and sundried prune products retained the vitamin C of the fresh fruit satisfactorily only when the fruit was dipped in lye in the usual commercial fashion before the sulphur

dioxide treatment. This is ascribed to better penetration by the protecting sulphur dioxide after the lye dipping.

All unsulphured products of both fruits whether sundried or dehydrated were without antiscorbutic value.

The dehydrated products both prune and apricot, retained the vitamin C more completely than did the corresponding sundried fruit.

The dehydrated and sundried apricots containing 450 to 500 or more parts of sulphur dioxide per million retained the antiscorbutic property more or less completely. With less than this amount, all products lost this property completely.

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A METHOD FOR REMOVING FAT SAMPLES FROM LIVE HOGS[1]

By E. L. SCOTT, *Assistant in Animal Husbandry, Department of Animal Husbandry*, and H. W. BLOCK, *Assistant Research Chemist, Department of Chemistry, Purdue University Agricultural Experiment Station*[2]

INTRODUCTION

In studying variations in the properties of fat as the fattening process advances it is often advisable to take successive samples from live animals. An experiment at the Purdue University Agricultural Experiment Station, conducted for the purpose of determining the influence of the degree of fatness of hogs on the quality of pork, provided the incentive for devising the sampling method described in this paper.

In early studies of fat development investigators used the method of killing "representative" individuals at different stages of development. In recent years workers have come to recognize the importance of the individuality factor. Scott [3] in 1920 emphasized specifically the great variation in the properties of the fat from different individuals kept on the same ration. Hankins and Ellis,[4] using the method of killing representative animals, also recognized individual variation and attempted to overcome its effect by using three animals at each killing.

Ewing and his coworkers [5] in 1919 described a method of removing fat samples from live hogs by means of a twisted clock-spring bit fitting inside a cannula. Later Scott [3] reported work in which he had removed fat samples from the rear part of the ham by making a slit about 2 inches long and taking out a piece of fat. A modification of this process in which the fat was removed through an incision in the back has been used recently at the University of Illinois.[6]

In agreement with the early work of Henriques and Hansen [7] it was found at the Purdue Agricultural Experiment Station [8] that the different layers of the adipose tissue of hogs yield fat of different constants. It was realized therefore that special precautions should be taken to insure proportionate amounts of the different layers in the sample when developmental studies are being made. This factor was considered of such importance as to warrant a careful study of the sampling process. It was felt that a sampling method should meet the following requirements: (1) Samples showing development of fat should come from the same individual; (2) they should be large

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[2] Appreciation is expressed to Dr. F. P. Mathews of the Veterinary Department of Purdue University for his helpful suggestions in connection with the technic of the operation.
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enough for the necessary chemical determinations; (3) they should be uniform in width and should extend clear through the fat tissue; (4) they should be taken from the same region, or from fat tissue of similar properties; (5) the sampling should be done in such a way as to cause the animal as little discomfort and setback as possible; (6) the incision should be made in a place where the chance for infection is slightest, and in such a way that the resulting wound will not be subjected to excessive strain; (7) the sample removed should not contain injected materials.

PRELIMINARY WORK

That the variation in the properties of the fat from samples taken at different locations on the fat backs of slaughtered hogs is so small as to be insignificant was the conclusion reached as a result of preliminary work at this station.[9] It was therefore considered that samples properly taken from the part of the back corresponding to the fat back would give comparable results.

At the beginning of an experiment conducted at Purdue University 80 pigs were sampled and the greater number of these were again sampled at intervals of 30 days for four successive samplings. Because of the results obtained in this and other experiments it is believed that the method herein described meets all of the requirements set forth above.

THE METHOD

PREPARING THE HOG

The hog is confined in a restraining crate of the hyperimmunization type, which is so designed as to hold him firmly in position. The top of the crate is open to permit the operator access to the back of the hog. A spot about the size of a man's hand directly over the loin is shaved, washed, and disinfected. By means of mercurochrome an oblong about 6 inches long and 2 inches wide is drawn on the shaved skin.

INJECTING THE ANAESTHETIC

With a metal hypodermic syringe the anaesthetic is injected intradermally along the mercurochrome line. The needle is inserted between the layers of the skin and is gradually pushed in to its full length while small amounts of the anaesthetic are being injected. When the entire skin oblong has been deadened a small quantity of the anaesthetic is injected between the fat tissue and the muscular layer at each end and side of the oblong and this completes the anaesthesia.

REMOVING THE FAT SAMPLE

When the skin is no longer sensitive the incision is made. An oblong piece of skin and fat about 4 or 5 inches long and thick enough to provide an adequate sample is removed. In making the incision it is advisable to keep well within the swellings caused by the injected liquid. Figure 1 illustrates the sampling method, gives a diagram of the sample removed, and shows the method of closing the wound. Care should be taken that the entire fat tissue is sectioned clear through to the muscular layer, and that the incision is so made as

[9] BLOCK, H. W. Op. cit.

to insure uniform width of the fat layers. If the layer of fat is thick the strip of tissue removed may be comparatively narrow, affording sufficient sample and yet permitting the wound to be closed easily.

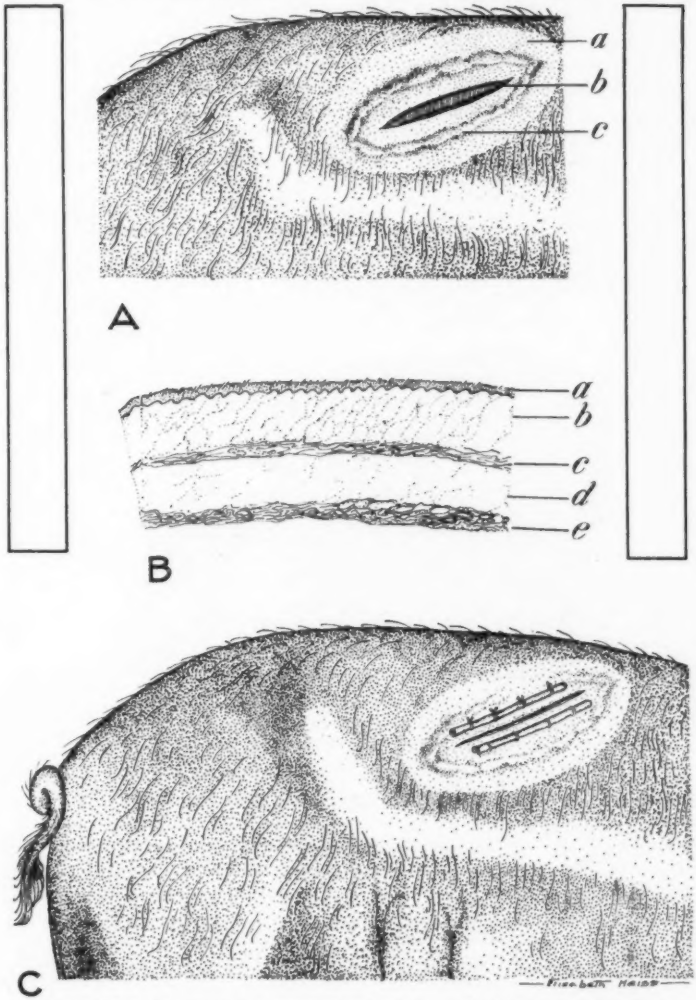

FIGURE 1.—Method of removing fat samples from live hogs. A, Incision for removing sample: *a*, Shaved area; *b*, incision; *c*, swellings where anaesthetic was injected. B, Diagram of removed tissue: *a*,Skin; *b*, subcutaneous fat layer; *c*, connective tissue; *d*, inner fat layer; *e*, connective tissue and blood vessels. C, Method of closing wound

CLOSING THE WOUND

By means of four quilled sutures, using half-curved cutting-edge suture needles and aseptic machinist's thread, the wound is closed. A thick-walled, $\frac{3}{16}$-inch rubber tubing on each side of the wound

proved effective in providing anchorage and in preventing the stitches from cutting through the skin. The wound is disinfected with 50 per cent alcohol and is then covered with pine tar. A little fish oil mixed in the tar renders the antiseptic dressing fly repellent.

HANDLING THE SAMPLE

After the sample has been removed it should be blotted free of blood, the skin taken off, and the network of blood vessels and connective tissue dissected from its ventral surface. Samples should be promptly placed in refrigeration and kept there until they can be delivered to the chemistry laboratory.

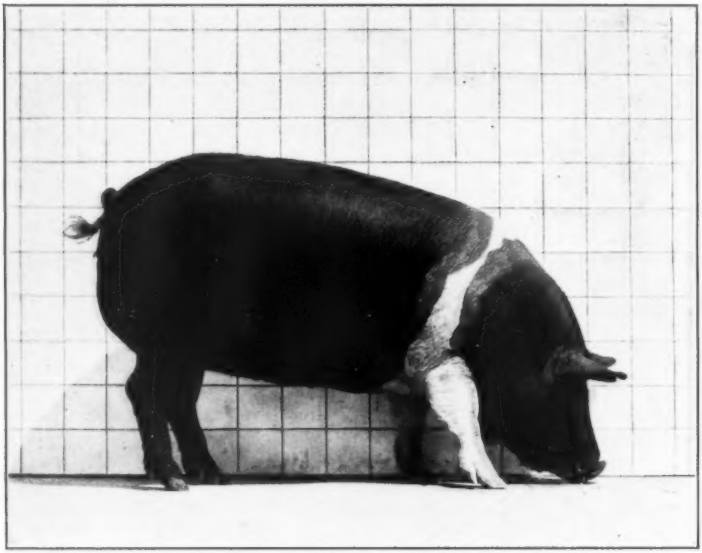

FIGURE 2.—An experimental hog from which four fat samples have been removed

ADDITIONAL OBSERVATIONS

Sterilization of instruments and materials was accomplished by boiling them in water. Fifty per cent alcohol proved to be a satisfactory antiseptic. An occasional case of infection occurred, but the hog invariably recovered.

Several different anaesthetics were tried. With magnesium sulphate the writers were unable to get proper anaesthesia. Administration of novocaine caused swelling and the animal would not resume feeding for about two days. The use of benzyl alcohol caused sloughing of the tissues. Finally a 1 per cent solution of apothesine (the hydrochloride of γ-diethylaminopropylcinnamate) was adopted as a convenient, efficient, and inexpensive anaesthetic.

The effectiveness of the anaesthetic depends upon the success of the operator in injecting intradermally. Black hogs (Poland Chinas and Hampshires) have a much thicker and less pliable skin than do

Yorkshires, for example. The latter are more difficult to anaesthetize, but skill in injecting the solution overcomes the difficulty.

Samples from the experimental hogs were taken adjacent to but on opposite sides of the backbone. In the early part of the experiment the incisions were made parallel to the backbone. Later it was found that if the incisions were made on the back running posteriorly and ventrally at an angle of approximately 45° to the backbone, good drainage was afforded and the healing was somewhat facilitated.

In the course of 8 to 10 days the wound appears to be healed, and most of the threads will give way and fall off with the rubber tubing. An individual from which four samples have been removed and the wounds healed is shown in Figure 2.

With one attendant to prepare the hogs, one operator to administer the anaesthetic and care for the samples, and another to remove the samples and dress the wounds, about 25 or 30 hogs can be sampled in eight hours.

This method has given good results with swine. With slight modifications it may be used in studying progressive fat development in other classes of meat animals.

A NEGATIVE CORRELATION OBSERVED BETWEEN THE NITRATE NITROGEN IN THE JUICE OF BEET LEAVES AND THE WEIGHT OF THE LEAVES [1]

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INTRODUCTION

Several workers have reported correlations between age or size of leaf and the physical or chemical properties of the leaf juice. Chandler (*1*),[2] working with apples and peaches, found the juice of the younger leaves to be less concentrated than that of older leaves, and Dixon and Atkins (*4*) found the same to be true of lilacs and other plants. Pringsheim (*11*), on the other hand, reported that the old leaves of Coleus yield a less concentrated juice than the younger leaves. Working with cornstalks, Hurd-Karrer (*9*) found the juice of the internodal sections to have a higher specific gravity nearer the tip of the plant. Eaton (*5*) noted that the juice from the older leaves of the cotton plant had a greater freezing-point depression than that from the young leaves. Chibnall (*2*) has attempted to compensate for the different sizes of leaves when analyzing plant tissue by segregating the leaves of different sizes and taking a representative proportionate aliquot weight.

During a series of determinations of nitrate nitrogen in various vegetable juices, the writer observed that the fluctuations in the quantity of this constituent were considerably larger than could be accounted for by the experimental error of the method. In the course of further investigation, a casual examination showed a significant negative correlation between the weight of beet leaves and the amount of nitrate nitrogen in the juice of these leaves. (Table 1.) In order to determine the significance of this observation, in the light of the results of the workers mentioned above, and to find an explanation for the phenomenon, the investigation herein described was carried out.

TABLE 1.—*Nitrate nitrogen in juice obtained from different weights of beet leaves*

Leaves	Average weight per leaf	Nitrate nitrogen in juice
Number	*Grams*	*Parts per million*
30	0.86	73
30	1.83	52
30	3.73	40
30	8.86	15

EXPERIMENTAL DATA

The beets considered in this paper were grown on $\frac{1}{30}$-acre market-garden plots at the Rhode Island Agricultural Experiment Station. These plots and their treatments have been described in detail by Hartwell and Crandall (*7*), and further discussed by Smith (*12*).

[1] Received for publication June 23, 1930; issued January, 1931. Published by permission of the director of the Rhode Island Agricultural Experiment Station as contribution No. 397.

[2] Reference is made by number (italic) to Literature Cited, p. 56.

The beets were planted April 13, 1928. The leaves used were taken on July 28 from plants growing on plots fertilized with an optimum amount of nitrogen, and were nearly mature.

Leaves were taken at random over the entire area of the plot from a number of individual plants estimated to be sufficient to obviate significant sampling errors. As soon as the leaves were picked they were taken to the laboratory, weighed, and the nitrate nitrogen in the juice was determined by the method of Gilbert (*6*). The data which exhibit the correlation are shown in Table 2.

TABLE 2.—*Correlation between nitrate nitrogen in beet juice and weight of leaves yielding juice*

Leaves	Average weight per leaf	Nitrate nitrogen in juice	Leaves	Average weight per leaf	Nitrate nitrogen in juice
Number	*Grams*	*Parts per million*	*Number*	*Grams*	*Parts per million*
10	6.5	9.8	40	4.7	42.5
20	5.7	13.8	49	3.7	35.9
20	5.9	27.6	50	4.3	31.1
29	4.0	42.5	60	3.8	51.8
30	4.6	42.0	115	3.2	57.6
40	4.2	34.7	115	3.2	70.4

Coefficient of correlation $r = -0.855 \pm 0.052$.

In calculating the coefficient of correlation the following formula was used:

$$rXY = \frac{\frac{\Sigma XY}{n} - \bar{X}\bar{Y}}{\sqrt{\frac{\Sigma(X^2)}{n} - \left[\frac{\Sigma(X)}{n}\right]^2}\sqrt{\frac{\Sigma(Y^2)}{n} - \left[\frac{\Sigma Y}{n}\right]^2}}$$

This formula, discussed by Hayes and Garber (*8*, *p. 43*), is especially applicable to small numbers of individuals. The probable error was calculated by the formula:

$$\text{P.E.}_r = 0.6745 \times \frac{(1-r^2)}{\sqrt{n}}.$$

The fact that the juice expressed from the midrib of a leaf is usually different in chemical composition from that expressed from the more actively metabolizing mesophyll tissue is well known to investigators. Few, however, have discussed this matter, preferring to eliminate an obvious source of error by removing the midribs before making the analysis. Among the many workers who have followed this procedure, Chibnall (*2*, *3*), Maskell and Mason (*10*), and Gilbert (*6*) may be mentioned.

It was learned early in the course of the investigation of the nitrate concentration in the juices of crop plants that the juice of the midribs of most leaves have a much higher concentration of this constituent than the juice from the remainder of the leaf. The data for beet leaves are given in Table 3.

TABLE 3.—*Nitrate nitrogen in fluids expressed from the midribs of beet leaves as compared with that from the remainder of the leaves*

[In parts per million]

Nitrate nitrogen in midribs	Nitrate nitrogen in leaves minus midribs
175	75
2,500	327
160	10
188	16
280	19

These figures suggest that the ratio of midrib tissue to the remainder of the leaf tissue varies with the size of the leaf, and if such is the case the relative amounts of nitrates present in the juice from large and small leaves would also vary. To determine the relation between the weight of the midrib and the weight of the remainder of the leaf both large and small beet leaves were gathered, the midribs were dissected out with a scalpel, and the two separate fractions weighed. Table 4 gives the results of these determinations.

TABLE 4.—*Relation of weight of midribs to weight of remainder of leaves for large and small beet leaves*

Leaves	Average length of leaves	Weight of leaves minus midribs	Weight of midribs	Weight of leaves minus weight of midribs / Weight of midribs
Number	*Cms.*	*Grams*	*Grams*	
15[a]	22	155.00	27.50	5.64
15[b]	11	24.91	6.25	3.98
20[a]	18	185.70	39.10	4.75
20[b]	8	17.82	4.72	3.77

[a] Large. [b] Small.

The differences in the ratio of the weight of the remainder of the leaf tissue to the weight of midrib tissue are comparatively large, and their magnitude is great enough, in the writer's estimation, to offset any crudity in the methods.

The negative correlation between the weight of leaf tissue and the nitrate concentration in the juice from these leaves is satisfactorily explained by the following facts:

(1) The juice of the midrib of the beet leaf contains considerably larger quantities of nitrate than does the juice from the remainder of the leaf.

(2) The ratio by weight between the leaf tissue less midrib to the midrib tissue is higher in large leaves, indicating a smaller proportion of midrib in these leaves.

It is obvious that when the tissue containing the greater quantity of nitrates is present in considerably smaller proportion of the whole leaf, the nitrate concentration in the juice of the leaf as a whole will be considerably less, thus accounting for the negative correlation observed.

It follows from the above explanation that in order to reduce the possibility of serious error in the sampling of comparative areas

of beets, it is necessary to choose leaves that are approximately the same size. It has been found that removal of the midrib tissue before analysis is necessary to obtain concordant results, since such high concentrations of nitrate nitrogen as exist in the juice of this tissue may introduce serious error.

This work is concerned only with the nitrate-nitrogen content of the juice of beet leaves. There is little doubt, however, that other constituents of the juice of beets, and perhaps of other plants, vary considerably with the weight of the leaf.

SUMMARY

A negative correlation was observed between the nitrate nitrogen in the juice of beet leaves and the weight of the leaves yielding the juice. It was also observed that juice from the midrib of the beet leaf contains considerably larger quantities of nitrate than does that from the remainder of the leaf. Finally, there is a smaller proportion of midrib by weight in large than in small leaves. Hence it is necessary to choose leaves of approximately the same size for analysis in order to avoid the possibility of a serious sampling error.

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